DRUG DISCRIMINATION

APPLICATIONS TO MEDICINAL CHEMISTRY AND DRUG STUDIES

EDITED BY
RICHARD A. GLENNON
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WILEY

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A JOHN WILEY & SONS, INC., PUBLICATION

Published by John Wiley & Sons, Inc., Hoboken, New Jersey.
Published simultaneously in Canada.

Library of Congress Cataloging-in-Publication Data:

Drug discrimination : applications to medicinal chemistry and drug studies / edited by Richard A. Glennon, Virginia Commonwealth University, Richard Young, Virginia Commonwealth University.
p. ; cm.
Includes bibliographical references and index.
ISBN 978-0-470-43352-2 (cloth)
1. Drug discrimination (Pharmacology) I. Glennon, Richard A., 1945- editor. II. Young, Richard, 1952- editor.
[DNLM: 1. Pharmacological Phenomena. 2. Discrimination (Psychology)–drug effects. 3. Discrimination Learning–drug effects. 4. Drug Discovery. QV 4]
RM302.7.D784 2011
615'.19–dc22

2010045225

oBook ISBN: 978-1-118-02315-0
ePDF ISBN: 978-1-118-02313-6
ePub ISBN: 978-1-118-02314-3

Printed in Singapore.

10 9 8 7 6 5 4 3 2 1

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PREFACE

This book is intended for the medicinal chemist and/or neuroscientist interested in investigations of neurochemical mechanisms that underlie the discriminative stimulus, or subjective, properties of drugs. Such studies in our laboratories have been focused on the idea that the effects of psychoactive agents are best expressed both in qualitative and quantitative terms. Our aim is to show that this approach has usefulness in the advancement of basic science, and is of practical value in the study of ethical pharmaceuticals and in the evaluation of drugs of abuse. For example, in certain instances, the stimulus potencies of drugs have been related to their human potencies. Furthermore, drug discrimination studies with animals have a human counterpart: drug discrimination studies with human subjects. The publication should serve as a ready reference for many investigators. They can refer to the book for details of the various methodologies commonly employed, available information applicable to numerous drugs and drug classes, discussions of how drug discrimination studies are designed and interpreted, and the limitations of the paradigm; most chapters are replete with actual data and illustrations.

During the past four decades, remarkable advances have been made in the study of drugs as discriminative stimuli. These will be described. In a number of ways, this book attempts to bridge the gap between earlier and newer topics in drug discrimination. The older and well-developed topics are related to newly developing areas. Our view is that the discriminative stimulus effects of drugs are a rapidly changing and expanding area of science. In no sense, however, can this book be regarded as some final description of the discriminative stimulus properties of drugs; rather, it must be viewed as a momentary state-of-the-science overview. The book provides historical background, presents a snapshot of where we are today (with opposing and controversial viewpoints where applicable), and includes some insight to where the field is headed. Indeed, the field evolves still. Thus, the book is a record of work done in this field, and provides results obtained not only by us but also by other investigators. The interested reader should find the book a good introduction to the background and procedures of drugs as discriminative stimuli, a useful introduction to the wealth of information that can be obtained from the paradigm, as well as being informative on the relatively complex processes of structure–activity relationships and mechanisms of drug action. Medicinal chemists need not be as fully versed in drug discrimination techniques as behaviorists to appreciate the utility of the drug discrimination paradigm

any more than behaviorists need be fully versed in topics fully understood by medicinal chemists—such as stereochemistry and drug design. Nevertheless, this book attempts to bridge these rather disparate but, in our opinion, complementary endeavors so that investigators on both extremes have a common vocabulary—so those designing and synthesizing novel chemical entities appreciate how their compounds can be evaluated, and so that those conducting the evaluations know what is behind the design and synthesis of the compounds they are examining. Chemists might find certain of the topics described herein to be rather trivial or mundane; behaviorists might find certain other chapters likewise. But, our intent is to bridge the gap between the various disciplines. What is common-knowledge to one might be a revelation to the other.

Studies on the subjective effects of drugs are of interest not only because they open up the possibility to gain new and accurate knowledge of the effects of many useful and experimental drugs, but also because they open up new vistas of how certain factors (e.g., dose and nature of training drug, presession injection intervals, route of administration, specific techniques, and animal species) can influence the qualitative and quantitative effects of drugs. The editors have attempted to organize the material in each chapter so that it is not described in isolation from other chapters; each chapter reflects, to some degree, the principles and/or concepts described in earlier chapters and, on occasion, is in anticipation of what will be described as issues in later chapters. Throughout the book, there are summaries of past research in the field as well as speculations or predictions of the future.

Inevitably, a book composed of chapters by multiple authors, with different styles and viewpoints, may vary in the interpretation of particular research findings; but no attempt has been made by the editors to impose conformity of viewpoint. The editors hope that differences in methodology or occasional inconsistencies in the interpretation of data will serve as a stimulus (no pun intended) for further research. Although the editors and invited authors may differ in their approaches to particular questions, or in their research techniques or orientation, all are dedicated to an objective and experimental evaluation of the discriminative stimulus properties of drugs.

In organizing the contents of this book, the editors decided early on that an attempt to provide exhaustive reviews of the stimulus properties of well-known drugs or of major drug classes was not our general goal; unfortunately, then, we were unable to invite many great practitioners of drug discrimination to contribute chapters (but perhaps in a future book?). Clearly, an attempt to explore these areas *in extenso* would have led to a multi-volume enterprise. Thus, the content of the book is restricted to subject areas generally not available elsewhere in a compact integrated form. The editors discuss basic principles of drug discrimination and the application of medicinal chemistry to drug discrimination studies in the first seven chapters. These chapters not only serve to highlight issues (and, sometimes, controversies) in drug discrimination but also might be helpful in other procedures and areas of behavioral pharmacology, medicinal chemistry, psychology, biology, physiology, and psychiatry. Thus, Part I (Chapters 1–7) describes the drug discrimination paradigm, the various methods and techniques employed, practical considerations, and examples of the general application of the method to investigate problems of interest. Chapters 1–3 should be of interest to those medicinal chemists not well versed in behavioral studies, whereas Chapters 4–7

might be particularly useful to those neuroscientists with limited training in stereochemistry, drug design, and drug development. Part II (Chapters 8–16) consists of invited chapters from investigators who have published extensively in the field of drug discrimination. They were invited to address specific topics or techniques that are of interest in drug evaluation and drug development. The editors are deeply indebted to these contributors. Their diligence and patience are warmly acknowledged as we arrived at a final publishable form of the book. On several occasions in Part II, material is referred to or included in order to point out its (as yet) incompletely realized promise as a field of study. It is hoped that others may continue to follow these promising studies.

From the editors' point of view, many contributions (scientific and otherwise) for Chapters 1–7 came from our students, technicians, postdoctoral fellows, and colleagues whose questions sometimes forced us to re-examine issues that we thought we had already understood, and whose research projects provided intellectual stimulation and (most of the time) fun. At this point, spanning more than 65 years of combined work by the editors, there are too many individuals to name—you know who you are (and many are cited in references that are provided)—who have helped us in clarifying some of the issues and provided the data that appear in Chapters 1–7. Last, but certainly not least, we both wish to acknowledge the aid of several individuals whose assistance was of great value to us: Jonathan Rose of Wiley Publishing, Dr. Malgorzata Dukat (experienced and published both in medicinal chemistry and behavioral studies) for her constructive comments on selected chapters, and Ms. Jennifer Degarmo, who was involved in the early phases of organizing the book, contacting authors, performing administrative tasks, and advising the editors. Finally, the editors acknowledge that their basic outlook of drugs as discriminative stimuli is, in many ways, a reflection of their numerous conversations with, and insights and suggestions from, Dr. John A. Rosecrans—a pioneer in this field, to whom we are greatly indebted. Our sense of gratitude is too great to be expressed simply.

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PART I

Part I is a detailed description of the drug discrimination paradigm, various methods and techniques employed, practical considerations, and examples of the general application of such methods to investigate problems of interest. Chapter 1 provides background/overview perspectives and specific commentary on the likelihood that a relationship may (or may not) exist between drugs as discriminative stimuli and drug abuse. Chapter 2 concentrates on specific methodological variables pertinent to studies of drug discrimination: 1) apparatus used, 2) subjects employed, and 3) a basic but relatively concise review of vocabulary for operant conditioning procedures. The beginning of Chapter 3 presents an impressive, but partial, list of drugs that have served as discriminative stimuli and then explores numerous issues, schemes, and tactics that confront investigators. Chapter 4 stresses the impact of chemical isomers when employed as training drugs and/or test agents. Chapter 5 illustrates how data obtained from drug discrimination studies are summarized and coherent structure–activity relationships (SAR) formulated. Chapter 6 provides examples of the mechanisms of action that are linked to the stimulus properties of certain drugs such as classical hallucinogens, amphetamine-related stimulants, designer drugs (e.g., MDMA, PMMA, α-ethyltryptamine), and therapeutic (e.g., antianxiety) agents. Finally, Part I closes with Chapter 7, which provides an overview of the relationships between drug discrimination studies and the development of agents as novel therapeutic entities or pharmacological tools.

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

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

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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

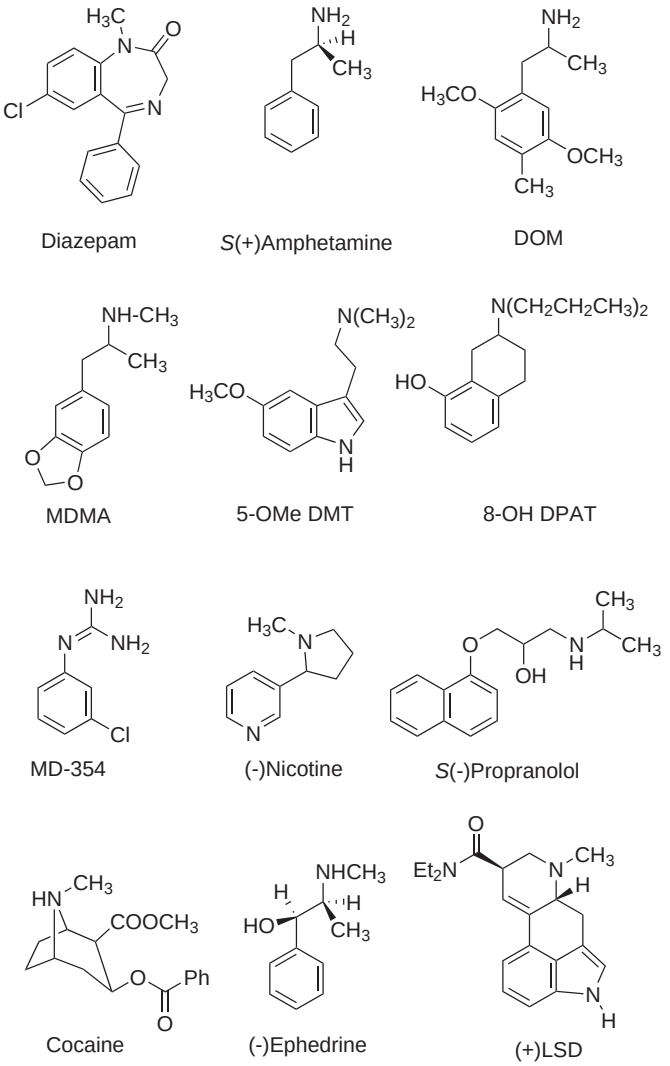

Figure 1-1. Chemical structures of some representative examples of agents that have been used as training drugs in our laboratories: diazepam, S(+)amphetamine, 1-(2,5-dimethoxy-4-methyl)phenyl-2-aminopropane (DOM), N-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDMA), 5-methoxy-N,N-dimethyltryptamine (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

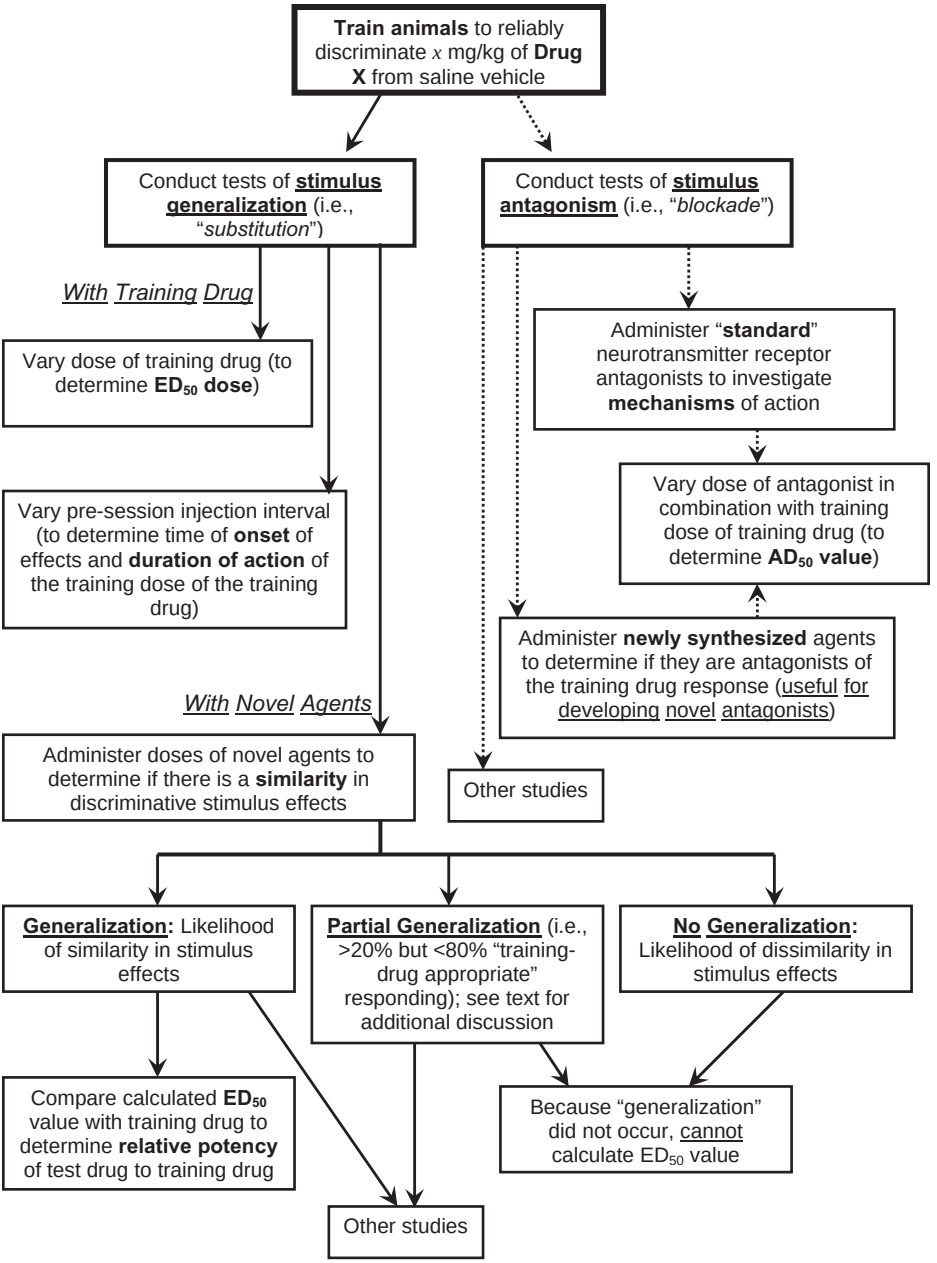

Figure 1-2. A simple schematic overview of some studies that can be conducted with animals trained to discriminate x mg/kg of a training drug, Drug X, from saline vehicle.

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).

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2

METHODOLOGICAL CONSIDERATIONS

A. APPARATUS

Early studies of drug discrimination used a single-choice T-maze device that required animals (usually rats) to choose between two alternatives on each of several trials. A typical T-maze experiment consisted of a start box with a door to restrain the animal, a stem that led from the start box to the choice point, and two alleys, one that led to a

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Edited by Richard A. Glennon, Richard Young.

left goal-box and one that led to a right goal-box. Another single-choice maze is the Y-maze in which the alleys to the goal box met the stem at a 45° angle instead of a 90° angle as in the T-maze. In typical maze experiments of drug discrimination, a rat might have been trained to turn to the right-alley (i.e., designated the drug-side for that rat) to obtain a food "reward," swim to an escape ladder, or escape a mild electric shock after administration of its dose of training drug, and to turn to the left-alley (i.e., designated the vehicle-side for that same rat) to receive reward, swim to a ladder, or escape shock after injection of vehicle [e.g., see 1–8]. Subjects, divided into two groups to control for position preference, learn to turn to the left after their dose of training drug and to turn to the right after vehicle; these conditions were reversed for the second group. A series of sessions would be conducted to train the animals and the treatment condition varied daily on an alternation or random sequence (e.g., Monday–vehicle, Tuesday–drug, Wednesday–drug, Thursday–vehicle, etc.). On a particular day, rats would be subjected to "massed trials," within a 30-minute session, for example, but only their response on the first trial within sessions was recorded because first-trial "fate" usually determined the animal's choice on trials that remained (i.e., *win, stay–lose, shift behavior*). In other words, the experimenter considered the animals' first response during the first trial of sessions, before any reinforcement (i.e., access to food, escape from shock, or access to ladder) was given, as a reflection of the degree to which they had learned to select the treatment-appropriate (correct) response.

Later, drug discrimination studies involved the use of a one-lever operant paradigm (see Chapter 11 by Negus and Banks) but eventually settled on a two-lever paradigm. Current studies of drug discrimination frequently employ a two-lever operant conditioning chamber. There were two reasons, at least in part, for the decline in use of the T-maze and the increased use of the 2-lever apparatus: 1) a consensus of thought among investigators of drug discrimination was that higher doses of a drug were needed to train rats in the T-maze than in the lever task, and 2) data analysis was limited to the animals' choice on only the first trial within sessions of the T-maze versus the animals' many presses of the levers in the two-lever operant chamber. Thus, if only the first response that the animals' made was considered, the evaluation of stimulus control was based on a very small sample of responses. For further discussion of the 1-lever *versus* 2-lever operant approach, see Chapter 11. At this time, drug discrimination studies are most often conducted in two-lever operant conditioning chambers (Figures 2-1 and 2-2). In particular, animal experiments of drug discrimination are conducted in chambers that eliminate or minimize the occurrence of extraneous events or conditions (e.g., loud sounds, bright lights, or temperature changes). The *set* of the chamber also is designed to make more likely the occurrence of a particular behavior. For example, when a (partially) food-restricted (i.e., "hungry") subject (rat, mouse, pigeon, or nonhuman primate) is placed in a chamber in which a lever or key is a prominent object there is increased likelihood that the animal will press the lever (or key), which will result in the presentation of reinforcement. Studies of drug discrimination are often conducted in standard two-lever operant chambers (e.g., Coulbourn Instruments, Whitehall, PA 18052, www.coulbourn.com; or MED Associates, St. Albans, VT 05478, www.medassociates.com) housed within light- and sound-attenuating outer chambers. Typically, one wall of each chamber is fitted with two levers (or pecking keys), also

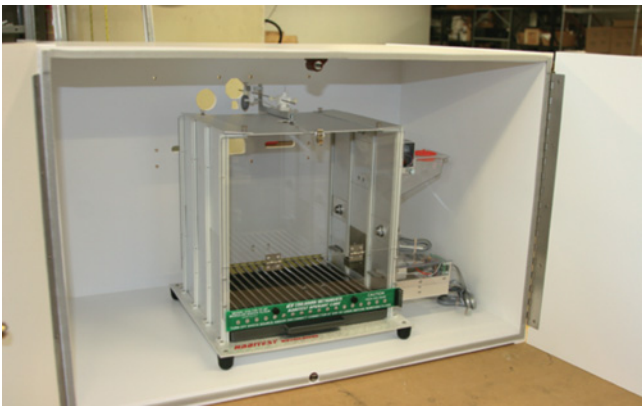

Figure 2-1. A bar-press apparatus, often called the Skinner box or operant chamber, that is commonly used in animal (i.e., rodent) studies of drug discrimination. Photo courtesy of Coulbourn Instruments.

Figure 2-2. An inside view of a typical (and basic) operant chamber, used for drug discrimination studies, that consists of two levers and a device centered between the levers for the presentation of reinforcement (inside lights may or may not be present). Pressing of a bar produces the presentation of reinforcement. Photo courtesy of Coulbourn Instruments.

referred to as *manipulanda*, and a device, centered equidistant between the levers, to present reinforcement. The reinforcement may be, for example, a 14-mg, 20-mg, 45-mg, or larger food pellet (e.g., Research Diets Inc., New Brunswick, NJ 08901, www.researchdiets.com; or Bio-Serv®, Frenchtown, NJ 08825, www.bio-serv.com), or sweetened condensed milk, or water, that is delivered in a 0.01-ml or 0.02-ml standard cup for rodents; other cup sizes are available from Coulbourn Instruments or Med Associates. An overhead house-light illuminates each chamber. Solid-state and

computer equipment are used to record lever presses, to program the delivery of reinforcement, and to record the number of reinforcements.

B. SUBJECTS

Table 2-1 shows that drug discrimination studies have utilized various animal species as experimental subjects. To date, the laboratory rat (*Rattus norvegicus*) has been used most often—probably because much is known about its anatomy, behavior, physiology, and neurochemistry. Investigators have bred many strains of rats for experimentation (mainly outbred stocks) and most are derived from the albino Wistar rat, which is still widely used. Other strains of rats are the Sprague-Dawley (relatively calm and easy to handle), Holtzman, Long-Evans (often used in obesity research), Lister, Lewis (noted for their docile behavior), and Fischer 344 (see summary of drug discrimination results with strains in Chapter 3, Table 3-9). The latter two strains of rats, for example, are inbred from the Sprague-Dawley rat and are compared frequently in their responses to drugs of abuse and situations of stress [e.g., see references 9–14]. Other inbred strains of rats also are available but are not as commonly used as, for example, inbred mice (*Mus musculus*). In addition, at this point in time, rat strains are generally not transgenic (i.e., have not been genetically modified) because the techniques of molecular genetics that work well in mice are apparently more difficult to apply to rats. Nonetheless, much of the genome of *Rattus norvegicus* has been sequenced. Currently, mice as subjects in drug discrimination experiments are appearing more frequently in the literature than in the past because various strains have been produced by a number of methods such as 1) mice bred through time-tested (traditional) methods; 2) transgenic mice, with foreign genes inserted into their genome; and 3) knockout mice, where a specific gene is rendered "inoperable" by techniques of molecular genetics to "knock out" a gene (see summary of results in Chapter 3, Tables 3-9 and 3-10). The genome of the mouse has

TABLE 2-1. Animal species used as subjects in drug discrimination experiments

Species	Number of Citations	Reference (example)
Cat, dog, guinea pig	1 each	Kilbey & Ellinwood [17] Cook et al. [18] Hudzik et al. [19]
Gerbil	24	Järbe et al. [20]
Human	267	Altman et al. [21]
Mouse	105	Snoddy & Tessel [23]
Monkey	528	Schuster & Brady [22]
Pig	3	Carey et al. [24]
Pigeon	363	Henriksson et al. [25]
Rat	2,716	Barry et al. [26]

Data obtained from citations in the Drug Discrimination Bibliography (www.drugrefs.org) and PubMed (www.pubmed.gov).

been sequenced and many mouse genes have human homologs. Thus, genetic manipulations in mice might form the basis to study the function (or lack thereof) of a gene, or to simulate a disease in humans. In the future, drug discrimination studies with rats will likely continue because of the enormous database of results that can be referenced, but mice will probably become more frequent as subjects because of the hundreds of inbred, outbred, transgenic, and knockout varieties that have been and/or will be created. Although rodents have been utilized most often in discrimination studies, nonhuman primates (monkeys) also have been employed, but they are expensive to acquire and maintain. It might be noted, because of their cost, studies with nonhuman primates typically involve relatively small sample numbers. The use and advantage of nonhuman primates as subjects are discussed by France and co-workers (Chapter 13). Last, but not least, drug discrimination studies that cite humans as subjects also are of interest (see Chapters 14 and 15 by Rush et al., and Perkins, respectively). In general, the procedures for humans are similar to those used for nonhuman animals, but are adjusted to the uniqueness of humans. For example, drugs are usually administered under double-blind conditions and money typically serves as reinforcement for correct responses. In addition, many human drug discrimination studies include self-rate scales, behavioral inventories, and/or questionnaires that gather data on subjective effects of the administered agent [e.g., see 15, 16]. Regardless of the animal subject, however, drug discriminations are learned under the pharmacological effect of different treatments (e.g., dose of drug versus non-drug conditions) that are linked to appropriate responses for the presentation of reinforcement.

C. OPERANT CONDITIONING

1. Historical Background and Terminology

Operant behavior is often termed "*the experimental analysis of behavior*" and is generally acknowledged to have begun with the publication of B.F. Skinner's book *The Behavior of Organisms* [27], which highlighted his laboratory research from around 1930 to 1937. Skinner's discoveries and analyses of the effects of consequences on behavior are his most powerful contributions to the study of behavior. Skinner and colleagues systematically manipulated the arrangement and schedule of events that preceded and followed behavior in many laboratory experiments from the 1930s to 1950s and derived basic principles of functional relations between behavior and events that continue to be used to this day (e.g., although dated, see Ferster and Skinner [28]). *Operant behavior* is any behavior whose frequency in the future is determined primarily by its history of consequences. *Consequences* affect the frequency of similar responses that will be emitted in the future under similar conditions. The response is selected, shaped, and maintained by the consequences that have followed it in the past. Moreover, an operant can take an unlimited range of forms. For example, a hungry rat may "act on" or "operate on" its environment and press a lever, which closes a switch and activates a food dispenser or liquid dipper to produce a pellet in a tray or liquid (e.g., sweetened milk) in a small cup, respectively. In such a situation, the rat's press of the

lever, which is followed by reinforcement (i.e., consequence), will probably result in an increased probability of the animal to press the lever in the future. In that situation, the animal's *operant response* is the press of the lever to close a switch, but it could have been the animal turning in circles, rearing (i.e., standing on the hind paws), or scratching. Basically, any response could have been used and been acceptable, as long as the animal could perform it.

Positive reinforcement (a.k.a. "*reinforcement*" or "*reward*") is the most fundamental principle of learning. *Positive reinforcement* involves an increase in responses as a function of the presentation of an event and, thus, is defined functionally rather than causally. The presentation of events such as food, water, or money may function as positive reinforcement and strengthen a response. In a complementary fashion, responses can lead to the termination of an event (e.g., switch off the noise of an alarm clock). *Negative reinforcement* occurs if a response increases as a result of the termination of an event (for reviews of laboratory and everyday situations of negative reinforcement see Hineline [29] and Iwata [30]). The removal of events such as shock, noise, or light may function as negative reinforcement and strengthen a response. In other words, the occasion of negative reinforcement is one in which the occurrence of a response will terminate, reduce, or postpone an event and, consequently, lead to an increase of that response in the future. Importantly, *positive and negative reinforcement have a similar effect on behavior in that both produce an increase in responses*. However, they differ with respect to the type of event that follows behavior. In both situations, an event (consequence) strengthens the behavior that preceded it. However, behavior maintained by positive reinforcement *produces* an event that was absent prior to a response, whereas behavior maintained by negative reinforcement *removes* an event that was present prior to a response. Thus, the critical distinction between positive and negative reinforcement is based on the presence or absence, respectively, of an event that occurs after a response.

Negative reinforcement is sometimes mistaken for punishment. That error is perhaps not unexpected because an often-used synonym for positive reinforcement is *reward*, which may lead to the conclusion that because the terms *positive* and *negative* are opposite, then it must follow that the opposite of reward is punishment. However the terms *positive* and *negative*, in reinforcement vocabulary, do not refer to something "good" and "bad" but to the presence versus removal, respectively, of an event that follows behavior. Another point of some confusion involves the acknowledgment that the events in both negative reinforcement and punishment are considered "aversive." In this regard, the same event may indeed serve as negative reinforcement in one context and as punishment in a different context, but both the nature and effect of the event on behavior are different. In a setting of negative reinforcement, an event that was present is terminated by a response, which leads to an *increase* in responses; in an instance of punishment, an event that was absent is presented after a response, which leads to a *decrease* in responses. For example, a response that terminates a mild electric shock would increase as a function of negative reinforcement, but a response that produces a mild electric shock would decrease as a function of punishment.

Negative reinforcement, at its most rudimentary level, involves an occasion of *escape*, in which a response removes (i.e., produces escape from) an ongoing event.

Keller [31], for example, provided an early study of escape when a rat was placed in a chamber; a bright light was switched on, and rats learned quickly to press a lever that turned off the light. In fact, people encounter escape situations every day, such as when they lessen or eliminate loud noises or shield their eyes from the sun. However, most behavior maintained by negative reinforcement is characterized by an opportunity for *avoidance*, in which a response prevents or postpones the presentation of an event. In either case, the presentation of an "aversive" event serves as a "motivational condition" for escape or avoidance and occasions a response that produced escape or avoidance from a similar situation in the past. A response that successfully terminates the situation will be strengthened. The latter conditions are illustrated in drug discrimination studies that involve discrete-trial procedures in which subjects can avoid or escape shock. For example, Schaefer and Holtzman [32] trained squirrel monkeys to discriminate the stimulus effects of intramuscular injections of 0.1 mg/kg of cyclazocine, a mixed opiate receptor agonist/antagonist, *versus* vehicle in a discrete-trial avoidance paradigm in which a (correct) response on one of two levers would prevent (i.e., avoidance) or terminate (i.e., escape) the delivery of an electric shock to animals' tail. Such discrete-trials procedures differ from other (e.g., hungry animal) procedures used in drug discrimination experiments in that the "motivation condition" is avoidance or escape from shock versus, for example, restriction of food or water (e.g., see Holtzman [33]).

2. Stimulus Control of Behavior

Behavior is influenced by events that occur prior to, and immediately after, its occurrence. The term *antecedent* refers to conditions or events that exist or occur prior to the behavior of interest. A *consequence* is the occurrence of an event that follows a behavior of interest. Some consequences, especially those that are immediate and relevant to a subject's current motivational condition, have a significant influence on future behavior. Consequences combine with antecedent conditions to determine what is learned. For example, in the aforementioned laboratory example of operant conditioning, a hungry (i.e., motivation condition) rat is placed in an experimental two-lever chamber, taught to press the levers, and, as a consequence, receives food pellets. Reinforcement of the rat's presses of the levers increases the frequency of lever pressing. Researchers who perform drug discrimination experiments make this simple task somewhat more complex by the introduction of another factor. That is, occasionally the rat is administered a dose of drug and receives food pellets only when the (now) drug-designated lever (for that rat) is pressed. On other occasions, that same rat is administered the vehicle (for that drug) and receives food pellets only when the vehicle-designated (alternate) lever is pressed. The dose of drug that exerts an effect and precedes the lever press is called a *discriminative stimulus*. A discriminative stimulus acquires a control function through association with events that occur immediately after behavior. The stimulus effects of a drug and "motivation conditions" (i.e., hunger of the rat) share two important similarities: 1) both events occur before the behavior of interest, and 2) both events have evocative functions (i.e., produce a behavior). In a typical two-lever drug discrimination experiment with a hungry animal, there is an interaction between these two antecedent events: 1) the motivation condition or state

of hunger, which evokes lever presses, and 2) the effect of the dose of drug or vehicle treatment, which evokes drug-designated or vehicle-designated lever presses, respectively. With some experience, the rat will concentrate its lever presses on the drug-designated lever in the presence of the effect of the dose of drug; few, if any, responses will be performed on the vehicle-designated lever. In other instances, the rat will settle its lever presses on the vehicle-designated lever after the administration of the vehicle (i.e., non-drug day); few, if any, responses will be performed on the drug-designated lever. In short, the animal has learned a drug versus vehicle discrimination. It has learned not only that the effect of the dose of drug and vehicle are simply different (which presumably it already "knew") but that the effect of drug and vehicle provide different information about the potential success of presses on a particular lever; for example, drug treatment indicates presses on the right-lever and vehicle treatment dictates presses on the left-lever. Thus, as the subject is exposed to training sessions and those aspects of the interactions of the training drug and neural sites that function as the stimulus effects of the dose of the training drug, the more likely the key pharmacological features will be recognized and the more accurate will be the subject's performance of the discrimination.

3. Drugs as Discriminative Stimuli

The discriminative stimulus effects of drugs have been established most frequently with operant conditioning procedures, learning situations in which subjects emit a response that is followed closely by reinforcement. As already noted, operant behavior is "controlled" by its consequences. In practice, operant conditioning is characterized by the study of behavior maintained by schedules of reinforcement, which are defined as the delivery of reinforcement to a subject according to some well-defined rule. In applications of drug discrimination, an animal's opportunity to press a lever under a schedule of reinforcement gives them, in effect, "*communication*" to the investigator of "how an agent affects their CNS." It is also noted that studies of the stimulus effects of drugs in humans have employed schedules of reinforcement and the patterns of response are generally similar to those obtained with nonhuman animals.

An animal's initial training in a two-lever operant task begins with "*magazine training*" or "*shaping*," which involves training them to eat from a food tray or drink from a dipper cup and, consequently, for them to learn that the noise made by the activation of a mechanical device indicates the presentation of "compensation." At the start of the study, the experimenter teaches the rats to press a lever for reinforcement with the technique of *shaping by successive approximation* (i.e., "*shaping*"). The latter procedure involves the reinforcement of behavior that may only be vaguely similar to the final desired response (i.e., lever-pressing); reinforcement continues for variations in behavior that come closer to presses of the lever. For example, the experimenter observes the rat and, rather than waiting for a lever press to occur, waits for some movement toward the lever, then delivers food. This strategy makes the animal's movements toward the lever somewhat more likely. The experimenter now waits until the rat moves closer to the lever before the presentation of food. Each delivery of food requires a behavioral action (i.e., movement), which is closer to a lever press than the

one before it. Within a relatively short period of time, the lever press response may be firmly established by this technique. The rat's behavior will have been so shaped that it will readily press a lever when put in the chamber. It is noted, however, that when large numbers of animals are used in an experiment, shaping can be a procedure that consumes a significant amount of time and requires much patience on the part of the experimenter. On the other hand, it is during this time that the animal typically adjusts to being handled. More often than not, rats initially show an "aversion" to being transported and handled. However, with sufficient exposure to the means of transportation and handling, they apparently become accustomed to it and, in comparison to their initial behavior, appear "gentled."

If a rat's every press of the lever is followed by reinforcement, then the animal is said to be under the schedule of *continuous reinforcement* (CRF). CRF is usually used to strengthen behavior, primarily during the initial stages of learning new behaviors. Some examples of everyday behavior that appear to mimic a CRF schedule include the activation of a faucet to obtain water or the insertion of money into a vending machine to obtain a product. In contrast, *extinction* (EXT) is demonstrated if reinforcement is withheld for a response and the frequency of that behavior decreases gradually to its prereinforcement level or ceases to occur. Thus, the occurrence of the response during EXT does not produce reinforcement. CRF and EXT can be considered endpoints for the availability of reinforcement but many "in-between" or intermittent schedules of reinforcement are possible in which some, but not all, occurrences of the behavior are reinforced. In such schedules of reinforcement, only selected occurrences of responses produce reinforcement.

If a hungry rat performs under a CRF schedule, then the number of reinforcements can become quite high and may lead to decreased responses over time because of satiation. However, every response is not required to be reinforced in order to maintain responding. That is, an animal can be reinforced intermittently (i.e., part of the time). The intermittent schedule can be based on a portion of responses or on a time interval. The two most common schedules of reinforcement are ratio and interval schedules, each of which can be fixed (unvarying) or variable (random or near random). In a ratio schedule, time is an irrelevant variable; number of responses is the factor that determines the delivery of reinforcement. As such, the organism's response rate does determine the rate (or number) of reinforcements. The more quickly the organism completes the ratio requirement, the sooner reinforcement will occur. In an interval schedule, number of responses is an irrelevant variable to when and how often the reinforcement will be delivered; elapsed time must occur before a single response is reinforced. Thus, reinforcement is contingent only on the occurrence of one response after the required time has elapsed. In a fixed schedule, the response ratio or the time requirement for reinforcement remains constant. In a variable schedule, the response ratio or the time requirement for reinforcement can change from one response to another. The combinations of ratio or interval and fixed or variable conditions define the four basic schedules of intermittent reinforcement: fixed ratio, variable ratio, fixed interval, and variable interval. In addition, differential reinforcement (DR) of particular rates (high = DRH, low = DRL) of behavior can occur as a variation of ratio schedules. In those schedules, the delivery of reinforcement is dependent on responses that occur at a rate either higher

than or lower than some predetermined criterion interval (see description below). The following section defines the four basic, DRH, and DRL schedules of intermittent reinforcement, provides an example(s) of each schedule, and describes some well-established schedule effects derived from basic research.

4. Basic Schedules of Reinforcement

a. Fixed Ratio If a subject must complete a specified number of responses before the presentation of reinforcement, then a fixed-ratio (FR) schedule of reinforcement is in effect. The FR 1 schedule indicates the availability of reinforcement after every response and is considered a special case that is termed continuous reinforcement or CRF (see above). In the case of an infrequent FR reinforcement schedule (i.e., number associated with FR is relatively high), a subject may be trained under a fixed ratio that is low initially but is increased gradually until performance under the high ratio value is attained (see discrimination training). Thus, a subject may eventually be trained under an FR 20 schedule, which indicates that 20 responses would be required to produce reinforcement. FR schedules produce high rates of response, and if ratio requirements are raised gradually over an extended period of time, extremely high ratio requirements can be reached. For example, rodents may press a lever or pigeons may peck a key hundreds of times per minute for food reinforcement under a fixed-ratio schedule. As such, responses that occur rapidly on an FR schedule will maximize the delivery of reinforcement because the quicker the rate of response, the greater the rate of reinforcement. The performer on an FR schedule produces a characteristic response pattern that can be described as follows: a) typically, after the subject's first response of the ratio requirement is fulfilled, completion of the required (i.e., remainder) responses occurs rapidly (i.e., with slight hesitations) between responses until b) the presentation of reinforcement, and, lastly, c) the participant does not respond for a period of time, which is termed a *post-reinforcement pause* (Figure 2-3). The size of the ratio influences the duration of the post-reinforcement pause; high ratio requirements produce long pauses and low ratios produce short pauses. There are many everyday examples of fixed-ratio schedules of reinforcement. People typically work rapidly with a fixed ratio because they receive reinforcement with the completion of the ratio requirements. Common examples are the piece-rate (or piecework) system in a factory, where the production of *n* units produces a payment, or farm workers who are paid to gather *n* pieces of fruit or vegetables to fill a box. Also, computer keyboarders often work on an FR schedule when they contract their services for specified amounts of work for specified amounts of pay.

b. Variable Ratio The participant on a variable ratio (VR) schedule of reinforcement must complete an irregular or varied number of responses to receive reinforcement. Moreover, the availability of reinforcement is defined statistically by a mean number of responses. In a VR 20 schedule, for example, a mean of 20 responses produces reinforcement. Reinforcement may occur after 2 responses, 40 responses, 6 responses, 16 responses, 36 responses, or *n* responses, but the average number of responses required for reinforcement is 20 (e.g., $2 + 40 + 6 + 16 + 36 = 100$; $100/5 = 20$). Typically, investigators use computers to select and program a VR sched-

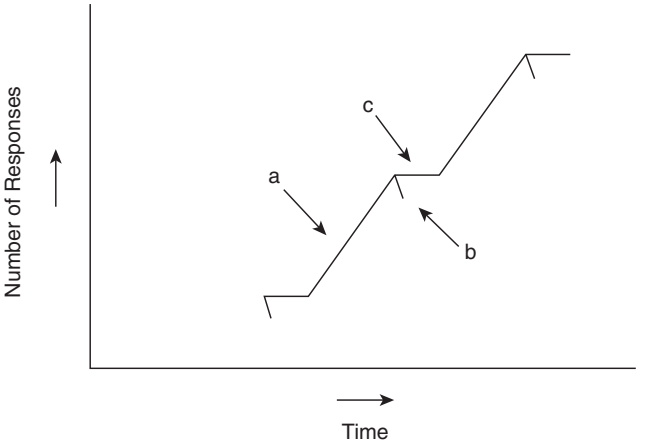

Figure 2-3. Response characteristics of subjects under a fixed ratio (FR) schedule of reinforcement include: (a) relatively "high" rate of response, (b) presentation of reinforcement (indicated by slash marks) upon completion of nth response and (c) after reinforcement, subjects usually pause ("post-reinforcement pause"); duration of pause can vary (see text).

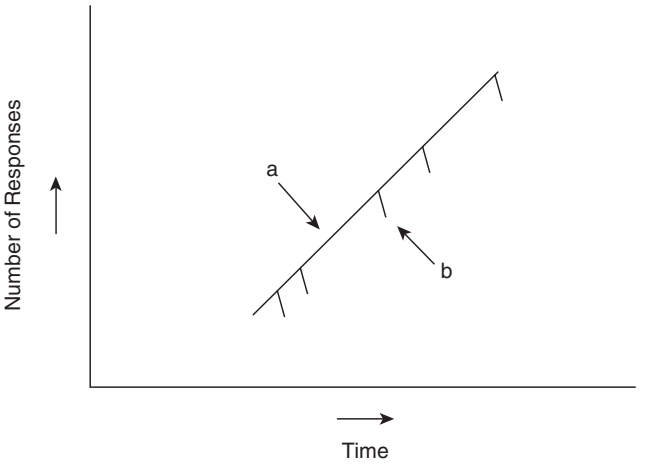

Figure 2-4. Response characteristics of subjects under a variable ratio (VR) schedule of reinforcement include: (a) relatively "high" and "steady" rate of response and (b) presentation of reinforcement (indicated by slash marks) upon completion of an irregular (variable) number of responses.

ule of reinforcement. The VR schedule tends to produce a) consistent, steady, and quick rates of response until b) the presentation of reinforcement (Figure 2-4). Such schedules are similar to FR schedules in that the size of the ratio number affects the rate of response; high ratio requirements are usually associated with high rates of response. VR schedules do not, however, usually produce a post-reinforcement pause. A classic

example of the variable-ratio schedule is gambling with a slot machine. Such devices are programmed to "pay off" (i.e., reinforce) only a certain proportion of the times they are played and a player cannot predict when the next activation of the machine will produce a win. The machine will average a certain schedule of nonorderly payoffs. The player may, for example, win 2 times in succession and then not win again for 35 or more plays and so on according to some programmed VR value. A theorist of operant learning would probably state that "a gambler's high rate of response on a slot machine is sustained by a variable-ratio schedule."

c. Fixed Interval The performer on a fixed interval (FI) schedule obtains reinforcement after their first response that follows a set duration of time. In an FI 3-minute schedule, for example, the first response that follows the elapse of 3 minutes produces reinforcement; premature responses—whether they are few or many—are not reinforced. The FI schedule is occasionally misinterpreted to indicate that the elapse of time *alone* is sufficient for the delivery of reinforcement. That is, reinforcement is mistakenly assumed to occur at the end of each fixed interval of time even if a response has not occurred. However, more time than the fixed interval can elapse between reinforced responses. Thus, reinforcement is available after the interval of fixed time has elapsed, and it remains available until the response. If the response occurs sometime after the elapse of a fixed interval, then that response is reinforced immediately and the set-up of another fixed interval is started with the delivery of that reinforcement. The FI cycle is then repeated until the end of the session. FI schedules tend to produce 1) a slow (i.e., "*pause*") to moderate rate of response during the early part of the interval, 2) an accelerated rate of response toward the end of the interval, and, lastly, 3) presentation of reinforcement (Figure 2-5). The duration of the FI affects the pause and the rate of response. Thus, fixed interval requirements that are long are usually associated with pauses that are long and rates of response that are relatively low. The pause of the FI schedule is similar to that of the FR schedule but the two schedules are distinguished by different characteristics of behavior that emerge under each schedule. A participant's responses under an FR schedule are emitted at a consistent rate until the completion of the ratio requirement, whereas responses under an FI schedule begin at a slow rate and accelerate toward the end of each interval, a pattern of responding that is referred to as the FI "scallop" (see segment of rounded curve in Figure 2-5). The appearance of a true FI schedule in everyday circumstances is difficult to identify but some situations do approximate the schedule. An example that is oftentimes described is the receipt of a paycheck for work on a scheduled basis (e.g., weekly, biweekly, or monthly) that is contingent on the first response (i.e., request to paymaster) on payday to produce the paycheck. In fact, however, the paycheck that is received requires many responses that are required—and constitute meaningful work—during the interval and eventually lead to issuance of the paycheck. In a true FI schedule, responses during the interval do not influence reinforcement. In fact, responses during the FI are irrelevant and such schedules have no deadlines for the response.

d. Variable Interval A subject who performs on a variable interval (VI) schedule of reinforcement receives reinforcement for their first correct response following

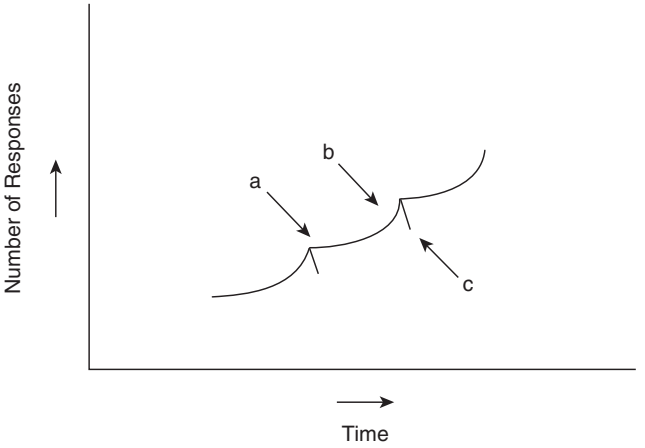

Figure 2-5. Response characteristics of subjects under a fixed interval (FI) schedule of reinforcement include: (a) relatively "low" rate of response ("pause") at the beginning of the interval, (b) increase in rate of response as the time for reinforcement approaches, and (c) presentation of reinforcement (indicated by slash marks) upon completion of first response after interval.

the elapse of various durations of time. Investigators describe VI schedules via an average (i.e., mean) interval of time before the subject's opportunity for reinforcement. In a VI 15-second schedule, for example, the average duration of intervals of time between reinforcement and the opportunity for subsequent reinforcement is 15 seconds. Thus, the actual time intervals in a VI 15-second schedule might be 8 seconds, 1 second, 3 seconds, 48 seconds, or *n* seconds, but the average (mean) elapsed time required for reinforcement is 15 seconds (e.g., 8 + 1 + 3 + 48 = 60; 60/4 = 15). Investigators typically use computers to select and program VI schedules of reinforcement as they do with VR schedules. VI schedules of reinforcement tend to produce a) low to moderate rates of response that are constant and stable with few pauses between responses until the b) presentation of reinforcement (Figure 2-6). In the VI schedule, like the FI schedule, the average duration of the time interval affects the rate of response; for example, mean intervals that are "long" are usually associated with lower rates of response. An example of a VI schedule of reinforcement occurs when one person telephones another person whose phone transmits a busy signal. This is a VI schedule because a variable interval of time is necessary for the second person to conclude the telephone conversation and hang up so that another call can be connected. After that interval, the first resend of the second person's number will probably produce an answer (the reinforcement). The number of responses (attempts) does not influence the availability of reinforcement in a VI schedule; no matter how many times the number that is busy is transmitted, the call will not be completed until the line is free. Also, the time interval is unpredictable in a VI schedule: the busy signal may last for a short or long time.

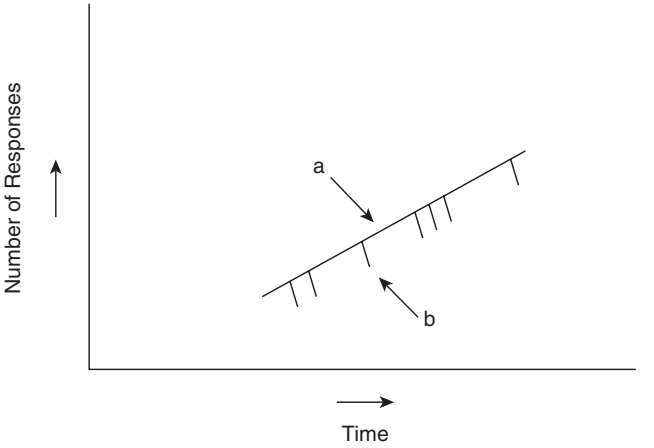

Figure 2-6. Response characteristics of subjects under a variable interval (VI) schedule of reinforcement include: (a) relatively "steady" rate of response and (b) presentation of reinforcement (indicated by slash marks) upon completion of a response after an irregular (variable) duration of time.

e. Variations of Basic Schedules

DIFFERENTIAL REINFORCEMENT OF LOW (DRL) OR HIGH (DRH) RATES OF RESPONSE In the aforementioned ratio schedules of reinforcement, the number of responses is the critical factor that determines the delivery of reinforcement. If an investigator decides that a subject will receive reinforcement only for *responses that are separated by a given or fixed duration of time*, then differential reinforcement of a particular rate of response is in effect. Thus, the performer's reinforcement is contingent on responses that occur at a rate that is either higher than, or lower than, some predetermined criterion. An inter-response time (IRT) describes the duration of time that occurs between two responses and is functionally related to rate of response: long IRTs indicate low rates of response and short IRTs indicate high rates of response. In a differential reinforcement of high (DRH) rates of response, a subject is reinforced whenever a response occurs *before* a criterion of time has elapsed. If the pre-stated criterion is 20 seconds (i.e., DRH 20 seconds), the participant's response produces reinforcement only when the IRT between responses is ≤20 seconds. In a differential reinforcement of low (DRL) rates of response, a subject is reinforced whenever a response occurs *after* a criterion of time has elapsed. If the predetermined criterion is 20 seconds (i.e., DRL 20 seconds), the participant's response produces reinforcement only when the IRT between responses is ≥20 seconds. If the participant responds earlier than 20 seconds, then there is no reinforcement and they have to wait 20 seconds from their premature response (without another response) before a response will produce reinforcement. In brief, DRH schedules "encourage" a high rate of response and DRL schedules "encourage" a low rate of response (Figure 2-7). In a drug discrimination experiment of note, Huang and Ho

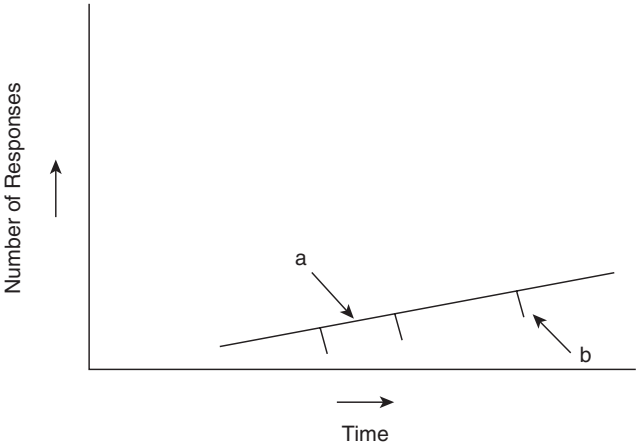

Figure 2-7. Response characteristics of subjects under a differential reinforcement of low rate (DRL) schedule of reinforcement include: (a) relatively "low" rate of response and (b) presentation of reinforcement (indicated by slash marks) whenever a response occurs after a criterion of time has elapsed. By reinforcing a low rate of responding, it is possible to get the almost flat curve of response shown here.

[34] successfully trained rats to discriminate the effects of the CNS *stimulant S*(+) amphetamine (0.8 mg/kg) from vehicle on a "seemingly incompatible" DRL schedule that required the animals to *wait* 15 seconds between responses (i.e., DRL 15 seconds).

5. Compound Schedules

In some operant conditioning schemes, researchers have combined the parts or elements of continuous reinforcement (CRF), the four basic schedules of reinforcement (FR, VR, FI, VI), variations of basic schedules differential reinforcement of rates (i.e., high or low) of responding (DRH, DRL), and/or extinction (EXT) to form compound schedules of reinforcement. Elements of compound schedules can occur 1) successively or simultaneously, 2) with or without events that signal a change-over or switch, or 3) as a contingency for reinforcement for each element independently or a contingency formed by the combination of all elements. Ferster and Skinner [28] provided a fairly clear description of compound schedules of reinforcement but, nevertheless, a discussion of such schedules can sometimes lead to confusion. Compound schedules of reinforcement are defined, described, and exemplified in the following section and a summary table (Table 2-2) is provided that (we hope) contains a relatively concise comparison and contrast of these types of schedules of reinforcement. The use of compound schedules of reinforcement in drug discrimination research springs, at least in part, from two reasons: 1) an assessment of rates of response or frequencies of reinforcement and 2) efforts to generate large numbers of responses to assess discriminative stimulus effects (i.e., percent drug appropriate responding). Thus, researchers might be interested in the

TABLE 2-2. Compound schedules of reinforcement: comparison and contrast

	Compound Schedule					
	Concurrent	Multiple	Chained	Mixed	Tandem	Second-Order
Number of distinct responses?	≥2	1	≥1	1	≥1	≥1
Is a signal linked to each schedule component?	Possibly	Yes	Yes	No	No	Possibly
Is there simultaneous (S) or successive (SS) presentation of basic schedules?	S	SS	SS	SS	SS	SS
Is reinforcement restricted to the final component of the basic schedule?	No	No	Yes	No	Yes	Yes
Is there reinforcement for independent components of the basic schedule?	Yes	Yes	No	Yes	No	No

All compound schedules of reinforcement contain ≥2 basic schedules of reinforcement.

stimulus effects of a dose of training drug *versus* vehicle when the presentation of reinforcement occurs under different and changing conditions; such assessments are often conducted with *concurrent* or *multiple* schedules of reinforcement (see below). Other researchers have employed compound schedules of reinforcement (e.g., *tandem* schedule) in drug discrimination tasks because the animal can make "many" nonreinforced responses on one (or both) levers prior to the presentation of reinforcement; that is, "more" responses prior to the presentation of reinforcement may be preferred in the calculation of percentage drug appropriate responding (i.e., the measure of the discriminative stimulus effect). In this regard, however, researchers have not established a consensus for a "preferred" number of responses. Indeed, the literature indicates that the fixed-ratio 10 (FR 10), fixed ratio 20 (FR 20), and (various) variable interval (VI) schedules of reinforcement are widely used and are thought to generate "acceptable" numbers of responses (see Chapter 3).

a. Concurrent Concurrent schedules of reinforcement occur when 1) two or more contingencies of reinforcement, 2) operate independently and simultaneously, 3) for two or more behaviors. People sometimes have opportunities for making choices among concurrent available events. For example, a graduate student receives a stipend from his/her department, contingent on performing daily work in the laboratory and tutoring undergraduate students. The student can choose when to do lab work and when to tutor, and the student can distribute their responses between these two simultaneously available schedules of reinforcement. In short, a concurrent schedule of reinforcement essentially matches two schedules of reinforcement against each other. In an example of a drug discrimination task, McMillan et al. [35] trained pigeons to discriminate the

effects of 5.0 mg/kg of pentobarbital from vehicle under a concurrent fixed-interval (FI) schedule of food presentation on which, after pentobarbital administration, responses on one key were reinforced with food under an FI 60-second component and responses on the other key were reinforced under an FI 240-second component. On other occasions, the pigeons were administered vehicle and the schedule contingencies on the two keys were reversed. Thus, the pigeons could distribute their responses between the two schedules of reinforcement after either treatment.

Signals (cues) can be used in compound schedules of reinforcement.

b. Multiple Multiple schedules involve two or more basic schedules of reinforcement in an alternation, usually random, sequence. In this situation, the basic schedules occur successively and independently. A *signal* is linked with each basic schedule, and that event is present as long as the schedule is in effect. In a lever-pressing task, for example, a subject may respond on an FR 10 schedule when a light is flashing and an FI 2-minute schedule when a bell is ringing. In a hypothetical example, a multiple schedule is highlighted in the following scenario in which a student may respond to basic facts about chemistry with their professor and also with their tutor. With the professor, the student responds to facts about chemistry during group instruction. The tutor then provides individual instruction and practice on the facts. This scenario follows a multiple schedule because there is one class of behavior (response to facts about chemistry), a signal for each contingency in effect (professor/tutor, group/individual), and different conditions for reinforcement (reinforcement is likely less frequent in group instruction).

c. Chained Chained schedules are similar to multiple schedules. A chained schedule differs from a multiple schedule in three ways. First, the basic schedules in a chain schedule always occur in a specific order, never in the random or unpredictable order of multiple schedules. Second, the behavior may be the same for all elements of the chain, or different behaviors may be required for different elements in the chain. Third, "reinforcement" for responses in the first element in the chain is the presentation of the second element; "reinforcement" for responses in the second element is presentation of the third element, and so on until all elements in the chain have been completed in a specific sequence. The last element typically produces reinforcement in the setting. In a lever-pressing task, for example, a subject may respond on an FR 10 schedule under a blue light condition and, when completed, the light then turns to green to indicate an FR 2 schedule and, when completed, the light then turns to red to indicate a VI 15-second schedule, etc. At the end of the series of schedules, reinforcement is finally given. In another example, a chained schedule is highlighted in the situation in which a student is involved in the multi-step (steps are assumed to occur in a specific order) synthesis of a target compound. The final outcome (target agent) is contingent on the completion of all steps. Thus, the synthesis follows a specific order of steps, different procedures or methods are used in each step, successful completion of one step leads to the next step and so on until the final step, which leads to the target compound for inclusion in a publication (i.e., reinforcement).

Signals are not used in some compound schedules of reinforcement.

d. Mixed A mixed schedule uses a procedure identical to that of the multiple schedule, except the mixed schedule has no signal that is linked with the independent schedules. For example, if a performer is on a mixed FR 20 FI 1-minute schedule, reinforcement sometimes occurs after the completion of 20 responses and sometimes occurs with the first correct response after a 1-minute interval from the preceding reinforcement. There is no signal to warn of the change in schedule.

e. Tandem Tandem schedules use a procedure identical to that of the chained schedule, except (like the mixed schedule) the tandem schedule has no signal that is linked with the elements in the chain. After a participant makes 20 responses on a tandem FR 20 FI 1-minute, then the first correct response following an elapse of 1 minute produces reinforcement. Some researchers have preferred a tandem schedule of reinforcement in drug discrimination tasks because the animal can make "many" nonreinforced responses on one (or both) levers prior to the presentation of reinforcement.

Sometimes signals are or are not used in compound schedules of reinforcement.

f. Second-Order Schedule Second-order schedules are another way to characterize a sequence of schedules. In these schedules, the behavior specified by a schedule contingency is treated as a unitary response that is itself reinforced according to some schedule of reinforcement. For example, reinforcement might be contingent upon the completion of three successive FI 2-minute schedules. A signal may or may not follow completion of each component; with a signal, a second-order schedule very much resembles a chain schedule; without a signal, the second-order schedule very much resembles a tandem schedule.

6. Schedules of Reinforcement and Drug Discrimination Studies

Table 2-3 lists the schedules of reinforcement that have been employed in drug discrimination experiments. To date, the schedules that have been used most, followed by their number of citations, are the fixed ratio (2,246), negative reinforcement (348), variable interval (208), and tandem (149).

There are several reasons that operant conditioning procedures are ideal to study the discriminative stimulus effects of drugs. First, the experimenter can, with relative ease, "shape by successive approximations" presses of levers (i.e., the response). Second, computer technology permits the automatic programming of the relationship of responses to their consequences and records accurately the subjects' response choices/rates. Lastly, and perhaps most importantly, operant conditioning procedures provide stable, yet sensitive, baselines for studying the discriminative stimulus effects of drugs over long periods of time; once trained, some animals may be on study for ≥2 years. In initial (and current) studies that reported various agents as discriminative stimuli (see Chapter 3 and Table 2-3), the fixed-ratio (FR) and variable interval (VI) schedules of reinforcement were used extensively and, consequently, animal data obtained under those schedules will be highlighted in Chapters 3–7. Moreover, current studies continue to employ the FR and VI schedules of reinforcement.

TABLE 2-3. Schedules of positive reinforcement, negative reinforcement, or punishment used in drug discrimination experiments[a]

Schedule	Citations	Reference (example)
Chained	1	Ferraro et al., [36]
Concurrent	14	Snodgrass & McMillan [37]
CRF	75	Shannon & Holtzman [38]
Differential Reinforcement of High (DRH) rates	0	
Differential Reinforcement of Low (DRL) rates	25	Cameron & Appel [39]
Fixed Interval (FI)	50	Poling & Appel [40]
Fixed Ratio (FR)	2,246	Kubena & Barry [41]
Mixed	0	
Multiple	5	Snodgrass & McMillan [42]
Negative Reinforcement	348	Schechter & Rosecrans [43]
Punishment	38	Poling & Appel [40]
Second-Order Schedule	27	McMillan [44]
Tandem	149	Kuhn et al., [45]
Variable Ratio (VR)	20	Leberer & Fowler [46]
Variable Interval (VI)	208	Kubena & Barry [47]

Data were obtained from citations in the Drug Discrimination bibliography (www.drugrefs.org) and PubMed.

[a]Some studies may be included in more than one category. For example, a two-lever discrete trial avoidance task may be counted under both negative reinforcement and CRF [e.g., 38]. Moreover, during animals' training, a punishment component may have been incorporated into basic schedules of reinforcement [e.g., 40].

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3

DRUG DISCRIMINATION: PRACTICAL CONSIDERATIONS

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

A. DRUGS AS DISCRIMINATIVE STIMULI

Table 3-1 lists some of the drugs, from various pharmacological and chemical classes, that have been shown to serve as discriminative stimuli. Classes of agents include antianxiety, sedative-hypnotic, narcotic analgesic, stimulant, hallucinogen, antidepressant, and antipsychotic drugs. In addition, the procedure has been used to study agents that exert various degrees of selectivity/action for acetylcholine, adrenergic, cannabinoid, dopamine, GABA, histamine, NMDA, norepinephrine, opioid, and serotonin neurotransmitter receptor systems. A survey (through the Drug Discrimination database of www.drugrefs.org) of authors and their department/employer affiliations revealed that investigators from many different disciplines (including pharmacology, psychiatry, psychology, medicinal chemistry, biology, and others) have embraced the methods of drug discrimination to study quite an array of psychoactive drugs and neurotransmitter receptor active agents. To date, agents that have been studied most (i.e., >250 citations), followed by their number of citations, include cocaine (545), *S*(+)amphetamine (436), morphine (390), ethanol (321), and (–)nicotine (273). Often, the use of training drugs and test drugs is limited by their commercial availability. Hence, medicinal chemists have a decided edge in this field because they can synthesize various agents of interest.

In most of the studies cited in Table 3-1, stimulus control was established *via* a two-lever operant conditioning task under an FR (e.g., FR 10 or FR 20) schedule of reinforcement. As such, subjects were taught to respond on one lever (e.g., right-side lever) when a dose of training drug was injected before a training session and on another lever (e.g., left-side lever) when vehicle was administered; correct responses were followed, intermittently, by the presentation of reinforcement (e.g., water, food pellet, sweetened milk). When the subjects' percentage of drug lever-appropriate responses that followed the administration of the dose of the training drug on drug-designated days was consistently distinct from that produced by the injection of vehicle on vehicle-designated days, the animals were assumed to have learned the discrimination task. Moreover, relatively good agreement exists between species (see Table 2-1) and/or schedules of reinforcement (see Table 2-3) on 1) whether or not a particular drug can

TABLE 3-1. A partial list of drugs that have been used as discriminative stimuli in drug discrimination experiments

Agent	Drug Class or Mechanism of Action	Citations	Reference (example)
Alprazolam	Antianxiety	9	Wettstein and Gauthier [1]
Amphetamine[a]	Stimulant	436	Schechter and Rosecrans [2]
Apomorphine	Dopamine receptor agonist	42	Colpaert et al. [3]
Arecoline	Muscarinic acetylcholine agent	18	Schechter and Rosecrans [4]
Atropine	Muscarinic antagonist	13	Barry and Kubena [5]
Baclofen	$GABA_B$ receptor agonist	6	Carter et al. [6]
Buprenorphine	Partial agonist (μ-opioid receptor)	12	Holtzman [7]
Bupropion	Antidepressant/ Smoking cessation	6	Jones et al. [8]
Buspirone	Antianxiety	21	Hendry et al. [9]
Caffeine	Stimulant	54	Carney et al. [10]
Cathinone	Stimulant	32	Goudie et al. [11]
Cholecystokinin	(Neuro) Peptide hormone	8	De Witte et al. [12]
Chlordiazepoxide	Antianxiety	116	Colpaert et al. [13]
Chlorpromazine	Antipsychotic	15	Goas and Boston [14]
Clenbuterol	β-Adrenergic agent	4	McElroy and O'Donnell [15]
Clonidine	α_2-Adrenergic/ $Imidazoline_1$ agent	17	Bennett and Lal [16]
Clozapine	Antipsychotic	66	Browne and Koe [17]
Cocaine	Stimulant	545	Colpaert et al. [18]
Codeine	Analgesic/Antitussive	20	Bertalmio et al. [19]
Cyclazocine	Opioid receptor agent	38	Hirschhorn [20]
Desipramine	Antidepressant	4	Shearman et al. [21]
Dextromethorphan	Antitussive	12	Holtzman [22]
Diazepam	Antianxiety	107	Young et al. [23]
Diphenhydramine	Antihistamine	5	Winter [24]
Ditran	Anticholinergic	8	Järbe et al. [25]
R(−)DOB[b]	Selective $5\text{-}HT_{2A}$ agonist	1	Glennon et al. [26]
DOI[c]	Classical hallucinogen	28	Glennon [27]
DOM[d]	Classical hallucinogen	80	Young et al. [28]
DPAT (8-OH)[e]	$5\text{-}HT_{1A/7}$ agent	75	Glennon [29]
Eltoprazine	$5\text{-}HT_{1A/1B}$ agent	8	Ybema et al. [30]
(−)Ephedrine	Adrenoceptor agonist	11	Gauvin et al. [31]
Ethanol	Stimulant/Sedative	321	Kubena and Barry [32]
Ethylketazocine	κ-Opioid receptor agonist	54	Hein et al. [33]

(*Continued*)

TABLE 3-1. (*Continued*)

Agent	Drug Class or Mechanism of Action	Citations	Reference (example)
Etorphine	Opioid receptor agonist	14	Herling and Woods [34]
Fenfluramine	Appetite suppressant	32	Goudie [35]
Fentanyl	Opioid analgesic	119	Colpaert and Niemegeers [36]
FG 7142[f]	Anxiogenic agent	5	Nielsen et al. [37]
Flumazenil	Benzodiazepine antagonist	49	De Vry and Slangen [38]
GHB[g]	GABA agent	29	Winter [39]
Haloperidol	Antipsychotic	18	McElroy et al. [40]
Heroin	Opioid analgesic	39	Corrigall and Coen [41]
Ibogaine	Anti-addiction agent	11	Schechter and Gordon [42]
Imipramine	Antidepressant	13	Schechter [43]
Isopropamide	Anticholinergic	1	Colpaert et al. [44]
Ketamine	NMDA receptor antagonist	49	Herling et al. [45]
Lorazepam	Antianxiety	44	Ator and Griffiths [46]
(+)LSD[h]	Classical hallucinogen	157	Hirschhorn and Winter [47]
mCPP[i]	Serotonin receptor agonist	18	Winter and Rabin [48]
MDA[j]	Designer drug	9	Glennon and Young [49]
MDL 100,907[k]	Selective 5-HT_{2A} antagonist	4	Dekeyne et al. [50]
MDMA[l]	Designer drug	78	Glennon and Misenheimer [51]
Mescaline	Classical hallucinogen	30	Hirschhorn and Winter [52]
Methamphetamine	Stimulant	72	Ando and Yanagita [53]
R(+)Methanandamide	$Cannabinoid_1$ receptor agent	8	Jarbe et al. [54]
Midazolam	Antianxiety	104	Garcha et al. [55]
MK-801[m]	NMDA antagonist	56	Sanger and Zivkovic [56]
Morphine	Opioid analegesic	390	Hirschhorn and Rosecrans [57]
Nalbuphine	Opioid analegesic	20	Walker and Young [58]
N-Allylnormetazocine	Sigma/κ-opioid receptor agonist	22	Shearman and Herz [59]
Nalorphine	Opioid receptor agent	9	Hirschhorn [20]
Naloxone	Antagonist (μ-opioid receptor)	31	Carter and Leander [60]
Naltrexone	Opioid receptor antagonist	83	Gellert and Holtzman [61]
(–)Nicotine	Nicotinic receptor agent	273	Schechter and Rosecrans [62]
NMDA[n]	NMDA receptor agonist	15	Amrick and Bennett [63]
Olanzapine	Antipsychotic	6	Porter and Strong [64]
5-OMe DMT[o]	Hallucinogen	25	Glennon et al. [65]
Oxazepam	Antianxiety	9	Hendry et al. [66]
PCP[p]	Dissociative anesthetic	200	Brady and Balster [67]

TABLE 3-1. (*Continued*)

Agent	Drug Class or Mechanism of Action	Citations	Reference (example)
Pentazocine	Opioid analgesic	27	Kuhn et al. [68]
Pentobarbital	Sedative	68	Herling et al. [69]
Pentylenetetrazol	GABA antagonist/ stimulant	134	Shearman and Lal [70]
Physostigmine	Cholinesterase inhibitor	11	Johansson and Jarbe [71]
PMMA[q]	Designer agent	12	Glennon et al. [72]
Pregnenolone	(Neuro) Steroid	8	Vanover [73]
S(−)Propranolol	β-adrenoceptor agent	1	Young and Glennon [74]
Quipazine	Nonselective 5-HT agent	19	White et al. [75]
Rimonabant	Cannabinoid CB_1 receptor antagonist	5	Järbe et al. [76]
Scopolamine	Muscarinic antagonist	25	Jung et al. [77]
Δ^9-THC[r]	Cannabinoid$_1$ receptor agent	112	Järbe et al. [78]
TFMPP[s]	Nonselective 5-HT agent	22	Glennon et al. [79]
Toluene	Solvent (abused by inhalation)	7	Rees et al. [80]
Triazolam	Antianxiety	47	Oliveto et al. [81]
Tripelennamine	H_1 receptor antagonist	7	Karas et al. [82]
Yohimbine	Nonselective α_2-adrenergic/5-HT agent	12	Winter [83]
Zolpidem	Hypnotic	26	Sanger and Zivkovic [84]

Data obtained from citations in Drug Discrimination bibliography (http://www.drugrefs.org) and PubMed (www.pubmed.gov).

[a]Almost all studies used *S*(+)amphetamine as training drug but a few used *R*(−)amphetamine or (±) amphetamine.

[b]*R*(−)-1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane.

[c]1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane.

[d]1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane.

[e]8-Hydroxy-2-(di-*n*-propylamino)tetralin.

[f]*N*-Methyl-9H-pyrido[5,4-b]indole-3-carboxamide.

[g]*gamma*-Hydroxybutyric acid, 4-hydroxybutanoic acid, sodium oxybate.

[h](+)Lysergic acid diethylamide.

[i]1-(3-Chlorophenyl)piperazine.

[j]1-(3,4-Methylenedioxyphenyl)-2-aminopropane.

[k](*R*)-(2,3-Dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol.

[l]*N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane.

[m](+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine maleate.

[n]*N*-Methyl-D-aspartic acid.

[o]5-Methoxy-*N*,*N*-dimethyltryptamine.

[p]1-(1-Phenylcyclohexyl)piperidine.

[q]*N*-Methyl-1-(4-methoxyphenyl)-2-aminopropane.

[r]Δ^9-Tetrahydrocannabinol.

[s]1-(3-Trifluoromethylphenyl)piperazine.

function as a discriminative stimulus and 2) results obtained with test (i.e., challenge) agents (see stimulus generalization tests below).

In addition, *three-choice* operant conditioning procedures have been employed, under various schedules of reinforcement, to characterize agents that exert different degrees of overlap in their 1) chemical structure, 2) mechanisms of action, and/or 3) pharmacological effect. In such procedures, subjects have been trained to discriminate two doses of the same drug *versus* vehicle or doses of different drugs *versus* vehicle to assess qualitative and/or quantitative differences in stimulus effects. In general, animal subjects cannot be trained to discriminate between two agents that produce highly similar stimulus effects. Thus, if a group of subjects can be trained to discriminate a dose of drug X *versus* a dose of drug Y *versus* vehicle, or two doses of drug X *versus* vehicle in a three-choice or -lever situation, then this may lead to a more precise characterization or delineation of the stimulus properties of the treatment conditions. For example, Table 3-2 shows that the three-choice procedure has been employed to evaluate 1) different doses of midazolam, morphine, hydromorphone, ethanol, or clozapine versus vehicle; 2) structurally related analogs such as the stereoisomers *R*(−)MDA versus *S*(+)MDA (see Chapter 4 for further discussion), *S*(+)amphetamine versus mescaline, hydromorphone *versus* butorphanol or naloxone, (+)LSD versus lisuride, morphine versus naltrexone, nalbuphine versus morphine or *S*(+)amphetamine versus MDMA from vehicle; 3) agents from the same pharmacological class such as morphine versus cyclazocine, pentobarbital or midazolam versus ethanol, buspirone versus diazepam, zolpidem versus triazolam, clozapine versus chlorpromazine, or ethanol versus

TABLE 3-2. Summary of three-choice drug discrimination studies in which subjects were trained to discriminate either two doses of the same drug versus vehicle or doses of different drugs versus vehicle

2.0 mg/kg Phencyclidine vs. 1.0 mg/kg Cyclazocine	White & Holtzman [85]
3.0 mg/kg Morphine vs. 0.3 mg/kg Cyclazocine	White & Holtzman [86]
5.6 mg/kg Morphine vs. 10 mg/kg Naltrexone	France & Woods [87]
10 mg Hydromorphone vs. 0.15 mg Naloxone	Preston et al. [88]
1.8 mg/kg Morphine vs. 10 mg/kg Morphine	Gauvin & Young [89]
0.08 mg/kg (+)LSD[b] vs. 0.04 mg/kg Lisuride	Callahan & Appel [90]

TABLE 3-2. (*Continued*)

10 mg/kg Cocaine vs. 0.1 mg/kg Haloperidol	Gauvin et al. [91]
3.0 mg Hydromorphone vs. 6.0 mg Butorphanol	Preston & Bigelow [92]
0.32 mg/kg Midazolam vs. 3.2 mg/kg Midazolam	Sannerud & Ator [93]
0.32 mg/kg Midazolam vs. 3.2 mg/kg Midazolam	Sannerud & Ator [94]
0.5 mg/kg *S*(+)amphetamine vs. 0.03 mg/kg Haloperidol	Caul et al. [95]
1.25 mg/kg *R*(−)MDA[a] vs. 1.25 mg/kg *S*(+)MDA	Young & Glennon [96]
1.0 mg/kg *S*(+)amphetamine vs. 12.5 mg/kg Mescaline	Baker & Taylor [97]
1.0 mg/kg *S*(+)amphetamine vs. 0.08 mg/kg (+)LSD[b]	Baker & Taylor [97]
10 mg/kg Pentobarbital vs. 1.0 g/kg Ethanol	Bowen et al. [98]
15 mg/70 kg Buspirone vs. 10 mg/70 kg Diazepam	Frey et al. [99]
5.6 mg/kg U-50,488H[c] vs. 5.6 mg/kg Morphine	Makhay et al. [100]
20 mg/70 kg Zolpidem vs. 0.5 mg/70 kg Triazolam	Mintzer et al. [101]
1.0 mg Hydromorphone vs. 4.0 mg Hydromorphone	Jones et al. [102]
0.35 mg/kg *S*(+)amphetamine vs. 0.033 mg/kg Haloperidol	Stadler et al. [103]
1.0 mg/kg *S*(+)amphetamine vs. 1.5 mg/kg MDMA	Goodwin & Baker [104]

(*Continued*)

TABLE 3-2. (*Continued*)

5.0 mg/kg Pentobarbital[d]	Li & McMillan [105]
vs.	
5.0 mg/kg Morphine	
vs.	
2.0 mg/kg *S*(+)amphetamine	
0.75 g/kg Ethanol	McMillan & Li [106]
vs.	
1.5 g/kg Ethanol	
5.0 mg/kg Pentobarbital	McMillan et al. [107]
vs.	
5.0 mg/kg Morphine	
5.6 mg/kg Nalbuphine	Walker et al. [108]
vs.	
3.2 mg/kg Morphine	
0.08 mg/kg (+)LSD[b]	Goodwin et al. [109]
vs.	
1.5 mg/kg MDMA	
3.0 mg/kg Midazolam	Porcu & Grant [110]
vs.	
1.0 g/kg Ethanol	
0.3 mg/kg *S*(+)amphetamine	Barrett et al. [111]
vs.	
0.03 mg/kg Haloperidol	
5.0 mg/kg Clozapine	Porter et al. [112]
vs.	
1.0 mg/kg Chlorpromazine	
1.25 mg/kg Clozapine	Prus et al. [113]
vs.	
5.0 mg/kg Clozapine	
20 mg *S*(+)amphetamine	Johanson et al. [114]
vs.	
0.75 mg mCPP[e]	
1.25 mg/kg Clozapine	Prus et al. [115]
vs.	
5.0 mg/kg Clozapine	
1.0 g/kg or 1.5 g/kg Ethanol	Baker et al. [116]
vs.	
300 mg/kg GHB[f]	

[a]1-(3,4-Methylenedioxyphenyl)-2-aminopropane.
[b](+)Lysergic acid diethylamide.
[c]*trans*-(±)-3,4-Dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl)cyclohexyl] benzeneacetamide methane sulfonate.
[d]4-Choice procedure.
[e]1-(3-Chlorophenyl)piperazine.
[f]*gamma*-Hydroxybutyric acid, 4-hydroxybutanoic acid, sodium oxybate.

GHB from vehicle; and 4) agents from different pharmacological classes such as phencyclidine versus cyclazocine, cocaine or *S*(+)amphetamine versus haloperidol, *S*(+) amphetamine or MDMA *versus* (+)LSD, pentobarbital versus morphine, or *S*(+)amphetamine versus mCPP from vehicle.

B. CHOICE OF DOSE AND PRE-SESSION INJECTION INTERVAL

An investigator must select a dose of training drug and pre-session injection interval (PSII) to employ in a drug discrimination study and these decisions may be straightforward or occur with various degrees of difficulty. There are, however, no guarantees of success. In one approach to these issues, an experimenter may decide quickly because an extensive database may already exist on one or more doses and the PSII of a training drug; *PSII* is defined as *the time interval between the administration of a dose of training drug and the start of the training session*. On the other hand, a database for a specific agent may not exist. In such a case, with rodents as subjects, for example, an investigator may collect preliminary data about the agent in an apparatus that measures changes in the animals' motor activity over time and then select a dose of training drug and PSII based on those results. Another approach may be to simply start with a "low" dose of an agent and begin training. If the animals' data for learning are not satisfactory, then the dose can be increased (e.g., doubled) and training is continued until a decision is reached that 1) acceptable results have been obtained, 2) a further increase(s) in the dose is needed, or 3) subjects will not achieve learning criteria for discrimination and the study is terminated. For example, the initial study of the discriminative stimulus properties of the purported 5-HT_3 receptor agonist 2-methylserotonin (2-Me 5-HT) began with 10 rats and a training dose of 1.0 mg/kg of 2-Me 5-HT (15 min PSII) *versus* saline vehicle [117]. Over a period of 7 months of training, animals did not learn (consistently) to respond on the appropriate lever after administration of either 1.0 mg/kg, 2.0 mg/kg, or 3.0 mg/kg of 2-Me 5-HT *versus* saline. The final (and eventually successful) training dose was 5.0 mg/kg; following 5 months of training sessions, subjects (eventually n = 4) consistently responded ≥80% of total responses on the 2-Me 5-HT-appropriate lever after injection of that dose of 2-Me 5-HT and ≤20% of their responses on the 2-Me 5-HT-appropriate lever after administration of saline. In a second example, the establishment of α-ethyltryptamine (α-ET), a designer drug of abuse, as a discriminative stimulus began with a training dose of 2.5 mg/kg using a 15 minutes PSII. After nearly one year of training at doses between 2.5 mg/kg and 5.0 mg/kg of α-ET, the dose was reinstituted at 2.5 mg/kg of α-ET, but the PSII was increased from 15 min to 30 minutes. With the longer (i.e., 30-minute) PSII, the animals quickly learned to discriminate α-ET *versus* saline vehicle (Figure 3-1).

Thus, conditions (i.e., 2.5 mg/kg of α-ET; 30-minute PSII) were eventually identified that resulted in a stable discrimination between α-ET *versus* saline [118]. Using these newly established conditions, a second group of animals was much more quickly trained to discriminate α-ET *versus* vehicle. A final example incorporates a *"fade-down"* procedure (mentioned in Chapter 1, Section E). Racemic MDMA has been used as a training stimulus in numerous drug discrimination studies and a typical training

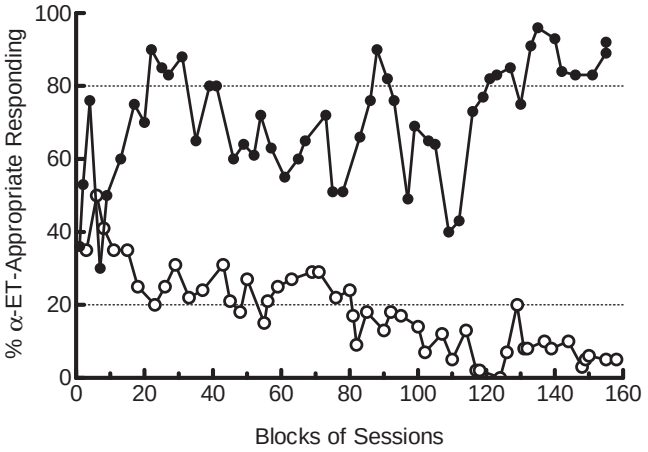

Figure 3-1. Learning curve of rats trained to discriminate α-ET versus saline vehicle. The study began with 2.5 mg/kg of α-ET as the training dose and a pre-session injection interval (PSII) of 15 minutes. Over time, the training dose and PSII were varied; training dose was increased to 3.5 mg/kg at the 18th block of sessions (2 sessions/block) and then 5 mg/kg after the 80th block of sessions. Subsequently, the training dose was decreased to 2.5 mg/kg, but the PSII was increased from 15 to 30 minutes at the 115th block of sessions. Closed circles represent the effect of α-ET and open circles represent response of saline vehicle (group means; S.E.M. not shown for purpose of clarity).

dose is 1.5 mg/kg. As such, the racemate training dose of MDMA consists of 0.75 mg/kg of *S*(+)MDMA and 0.75 mg/kg of *R*(–)MDMA. Therefore, studies were begun at 0.75 mg/kg of each isomer in separate groups of rats with the expectation that the dose of at least one of these two isomers would function as a discriminative stimulus [119]. Over time, however, the training dose of *R*(–)MDMA was incrementally increased to 1.5 mg/kg (with no success) and then 2.5 mg/kg (with success). Once responding was consistent, a "fade-down" procedure was employed to decrease the training dose of *R*(–)MDMA to 2.0 mg/kg and later to 1.5 mg/kg; however, the latter dose produced somewhat inconsistent results in the animals but a slight increase in the dose to 1.75 mg/kg produced stable performance (Figure 3-2). In comparison, animals readily learned an *S*(+)MDMA *versus* saline discrimination when the training dose was gradually increased from 0.75 mg/kg to 1.5 mg/kg [119]. Taken together, the results suggest that racemic MDMA is more effective as a training drug than half the dose of either optical isomer.

Lastly, a fourth strategy for the selection of dose and PSII is to rely on data obtained in stimulus generalization tests. For example, rats can reliably discriminate the stimulus effects of 1.0 mg/kg of DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) *versus* vehicle [120]. In tests of stimulus generalization, the DOM stimulus generalized (≥80% DOM-appropriate lever responding) to 0.2 mg/kg of *R*(–)DOB (*R*(–)-1-(4-bromo-2,5-dimethoxyphenyl)-2-aminopropane). In a subsequent study, a separate group of animals was trained to discriminate *R*(–)DOB at that dose (i.e., 0.2 mg/kg) *versus* saline vehicle [26].

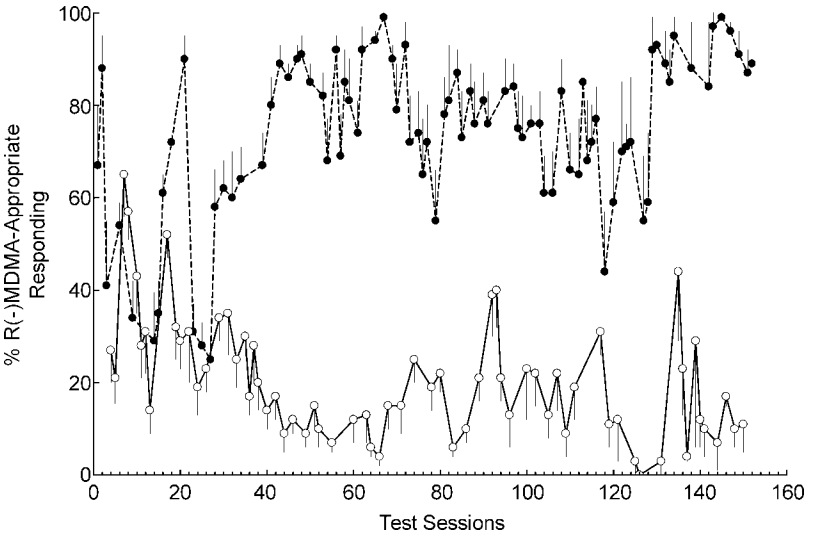

Figure 3-2. Learning curve of rats trained to discriminate R(–)MDMA versus saline vehicle. The study began with an R(–)MDMA training dose of 0.75 mg/kg. Over time, the training dose was incrementally increased to 1.5 mg/kg at the 15th training session and then to 2.5 mg/kg at the 28th training session. Once responding was fairly consistent, a "fade-down" procedure was employed to decrease the training dose to 2 mg/kg at the 57th training session and later to 1.5 mg/kg at the 73rd training session. Due to the instability of the latter dose to reliably maintain drug-appropriate responding, the training dose was increased to 1.75 mg/kg at the 128th training session. Closed circles represent the effect of R(–)MDMA and open circles represent response of saline vehicle (group means).

C. DISCRIMINATION TRAINING PROCEDURE

The most widely used method in drug discrimination studies involves subjects who are taught to distinguish the effect of a specific dose of training drug *versus* vehicle under a positive schedule of reinforcement. The survey that follows in Chapters 3–7 describes some training and test results (i.e., stimulus generalization, time course, stimulus antagonism, SAR, stereochemistry, metabolism) from rats trained to discriminate the following agents *versus* vehicle: diazepam (3.0 mg/kg, i.p.) or pentobarbital (5.0 mg/kg, i.p.) *versus* vehicle (one drop of Tween 80 per 10 ml distilled water for suspension of diazepam or saline for solution of pentobarbital) under a *fixed ratio 10* (FR 10) schedule of reinforcement; *S*(+)amphetamine (1.0 mg/kg, i.p.), DOM (1.0 mg/kg, i.p.), MDMA (1.5 mg/kg, i.p.), *S*(–)propranolol (5.0 mg/kg, i.p.), (–)nicotine (0.4 mg/kg or 0.6 mg/kg, s.c.), cocaine (8.0 mg/kg, i.p.), (–)ephedrine (4.0 mg/kg, i.p.), 8-OH DPAT (0.1 mg/kg, i.p.), (±)MDA (1.5 mg/kg, i.p.), *S*(–)methcathinone (0.5 mg/kg, i.p.), or 5-OMe DMT (1.5 mg/kg or 3.0 mg/kg, i.p.) *versus* saline under a *variable interval 15-second* (VI-15 second) schedule of reinforcement. For these studies, albino Sprague-Dawley male rats (Charles River Labs, Wilmington, MA), experimentally naïve, that

weighed 300 g to 350 g at the start of an experiment were used. Rats were housed individually and had free access to water, but were gradually food restricted to approximately 80% of their (growing) free-feed weights before the beginning of training. The colony room was kept at a constant temperature (approximately 21° to 23°C) and humidity (~50%); lights were turned on from 0600 to 1800 hours.

In the aforementioned studies of drug discrimination, initial training sessions were preceded by injection of either the dose of training drug or vehicle with only the treatment–appropriate lever present (i.e., left- or right-side lever). A pre-session injection interval (PSII) of 15 minutes was used; animals were kept in their home cages for this interval. The route of administration and the PSII for each drug and its vehicle were chosen on the basis of their known pharmacokinetic properties and/or behavioral effects. Training sessions were of 10 minutes (diazepam study) or 15 minutes (all other training drugs) duration, 5–7 days per week. For a particular session, just one of the two levers (i.e., the treatment-appropriate lever) was programmed to present reinforcement; presses on the incorrect lever had no programmed consequence. For half of the rats in each group, responses on the right-side lever were reinforced after administration of the dose of drug while responses on the left-side lever were reinforced after vehicle administration; lever response conditions were reversed for the remaining rats in each group. In addition, lever assignments for a particular operant chamber were alternated (e.g., 1st animal in chamber #1 was assigned the left-side lever as drug lever and the right-side lever was the saline lever, 2nd animal in chamber #1 was assigned the right-side lever as the drug lever and the left-side lever was the saline lever, etc). The latter tactic is important because of the finding that rodents may learn to use olfactory hints (i.e., cues) that remain on the levers by animals that preceded them; furthermore, the levers should be wiped clean at the start of each session to prevent olfactory cues [121]. In addition, the dose of training drug or vehicle was administered on a random schedule with the constraint that no more than two consecutive sessions with the drug or vehicle could occur; an equal number of sessions are scheduled with drug and vehicle. The experimenter may note that initial injections of the dose of training drug typically hinder or disrupt the animals' pressing of the drug-designated lever. Animals develop (behavioral) tolerance to the disruptive effects of the drug and will, over time, perform the task [e.g., see 122]. Animals do not, however, develop tolerance to the stimulus effect of the training dose of the training drug. If such tolerance does develop, then the dose of the training drug would not continue to serve as a discriminative stimulus and the animals' performance would decline significantly.

D. DISCRIMINATION DATA

1. Quantal or Quantitative (Graded) Analysis

Drug discrimination data can be expressed as a quantal and/or quantitative (graded) measure; each expression provides an indication of lever preference prior to, up to, or without the presentation of the first reinforcement (e.g., in an FR 10 schedule of reinforcement). *In a quantal approach, the lever that is pressed 10 times (first) is designated*

as the "selected" lever, which can be either the drug-designated lever or the vehicle-designated lever. A subject's response is considered all-or-none, such that discriminative stimulus effects are either like or not like the dose of training drug, with no possibility of an intermediate or partial response. Moreover, the quantal measurement of discriminative stimulus effects is the percentage of subjects that select the drug-designated lever as their selected lever (i.e., the first lever that was pressed 10 times). Investigators who employ the quantal approach will report data of either "0% of subjects selecting drug lever" or "100% of subjects selecting drug lever." Dose response data are produced from averages of results from subjects that select one lever (i.e., either 0% or 100% score) with the results of subjects that select the other lever (i.e., either 100% or 0% score) at certain doses. Moreover, once dose-response functions have been determined for two or more agents, they can be compared in terms of their potency to produce a particular discriminative stimulus effect. In a quantal approach, the potency of drugs is estimated by use of the relation between doses and the proportion of subjects responding. Those ratios do not necessarily have a linear relationship and it is customary to assume a suitable distribution for appropriate linearization. For example, potency is often characterized by the median effective dose 50%, symbolized by ED_{50}, or median antagonism dose 50%, symbolized by AD_{50}. These are calculated doses that, on an average, produce the desired response in 50% of subjects. In other words, an ED_{50} or AD_{50} value is calculated to indicate the percentage of individuals who show the desired effect (i.e., *drug-like effect in 50% of subjects*) at a particular dose level. In the calculation of an ED_{50} or AD_{50} value, the parameters of the distribution are estimated (first) from the data via, for example, the maximum likelihood estimation or the minimum χ^2 methods. The assumption of a normal distribution for percentage of subjects responding leads to what is known as *probit* scores or analysis (mean of 5, standard deviation of 1) and the assumption of a log-dose (logistic) distribution leads to *logit* analysis. For the interested reader, probit and logit analysis is discussed in more detail by Litchfield and Wilcoxon [123] and Tallarida [124].

In comparison, subjects' responding is quantitative or "graded" when their data are presented as percent drug-appropriate responding, which is obtained by dividing their number of responses on the drug-designated lever by their total number of responses on *both* levers at the time that the 10th response (e.g., in an FR 10 schedule of reinforcement) is performed on either lever; this fraction is then expressed as percent drug-appropriate responding. When a quantitative approach is employed, an ED_{50} or AD_{50} value for a dose-effect function is typically determined with linear regression (least squares method) analysis from the linear portion (i.e., typically middle section) of the curves [see 124–126]. In this analysis, the calculated ED_{50} or AD_{50} value is a calculated estimate of the dose at which the subjects would be expected to make *50% of their responses on the drug-appropriate lever* [126].

The calculation of an ED_{50} or AD_{50} value by the quantitative or the quantal method that includes one or more "low-end' or "high-end' values, especially without intermediate data, will affect the slope of a line and, consequently, skew the calculated value. In Figure 3-3, for example, separate ED_{50} values were calculated from one set of data. The calculation of the ED_{50} value (6.3 mg/kg) from "open squared" data included multiple "low-end" values, which produced an ED_{50} value that appeared to be more

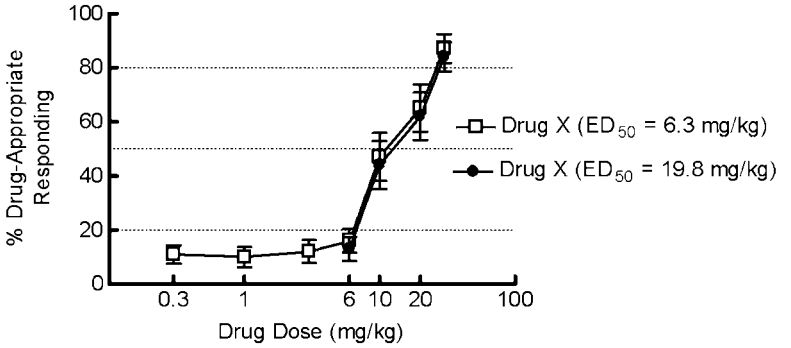

Figure 3-3. Separate ED_{50} values calculated from one set of hypothetical data that either included or did not include multiple "low-end" data points. The calculation of the ED_{50} value (6.3 mg/kg) associated with open squared data included multiple "low-end" values, which produced an ED_{50} value that appeared to be more consistent with the hypothetical dose of drug that produced vehicle-like responding (i.e., ~20% drug-appropriate responding). In comparison, the determination of the ED_{50} value (19.8 mg/kg) associated with closed circles (without the low-end points) revealed that it was much closer to the hypothetical dose of drug that produced 50% drug-appropriate responding.

consistent with vehicle-like responding (i.e., ~20% drug-appropriate responding). In comparison, the ED_{50} value (19.8 mg/kg) determined by "closed circle" data did not include the lower-end values but instead concentrated on data obtained from doses in the middle section of the curve. Visual inspection of the latter ED_{50} value, in comparison to the former ED_{50} value, revealed that it was much closer to the hypothetical dose of drug that produced 50% drug-appropriate responding.

Importantly, a given agent in drug discrimination studies, unlike most other pharmacological assays, can have more than a single ED_{50} value. Thus, *calculated ED_{50} values are relative to the dose response that is associated with the dose of training agent*. Typically, as the training dose of the training drug increases, the ED_{50} value of the training drug increases, and the ED_{50} value of agents to which the training drug generalizes also can increase. For example, Waters et al. [127] calculated an ED_{50} value of 0.15 mg/kg for (±)amphetamine in a group of animals trained to discriminate 0.3 mg/kg of (±)amphetamine from vehicle; in a second group of animals trained to discriminate 2.5 mg/kg of (±)amphetamine from vehicle, the ED_{50} value was approximately 1.0 mg/kg of (±)amphetamine.

Further discussion of the attributes of quantal and quantitative analyses of data from drug discrimination studies can be found in reports by Colpaert [128], Stolerman [129], and Colpaert [Chapter 16]. Generally, both approaches to data management are thought to produce similar results and conclusions [see 35, 130–133]. Regardless of whether a quantal or quantitative approach is employed, however, *ED_{50} values should be accompanied by a statement of the error of the estimated value such as the probability range or (±) 95% confidence limits*. Indeed, ED_{50} or AD_{50} values of different drugs, or dose-response shifts of one drug, can be compared for statistically significant differ-

ences with a comparison of 95% confidence intervals; interval overlap indicates that the values are not statistically different and no interval overlap indicates that they are statistically different.

Lastly, in drug discrimination experiments, interactions between the stimulus effects of drugs can be quantified and characterized in reference to dose additive effects *via* isobolographic analysis, which was originally developed by Fraser [134] and refined and/or reviewed by various investigators [e.g., 135–138]. An isobologram is a plot of constant effect for two or more drugs given in combination. An isobole is an iso-effect curve that represents the set of all drug combinations that have the same potency, usually measured as the ED_{50} values for a training drug and an agent that produced a training drug-like effect, plotted on a graph whose axes are the dose-axes of the individual drugs. In Figure 3-4, for example, the straight line (i.e., plot 1 isobole curve) that connects the ED_{50} dose of drug X on the x-axis and the ED_{50} dose of drug Y on the y-axis is the line of *additive effect* because it represents combinations of doses of the two drugs that are equipotent. Thus, an additive effect is stated when the combined stimulus effects of two drugs is the simple algebraic sum of their individual actions.

When points that represent the stimulus effects of a combination(s) of doses of the drugs lie below and to the left of the line (i.e., plot 2) of additive effect, the interaction is termed *supra-additive* or *synergistic*. Thus, synergism is stated when selected doses of each of two drugs have measurable stimulus effects and, when combined, their effects are greater than the algebraic sum of the individual actions of the two drugs. On the other hand, when points that represent the stimulus effects of a combination(s)

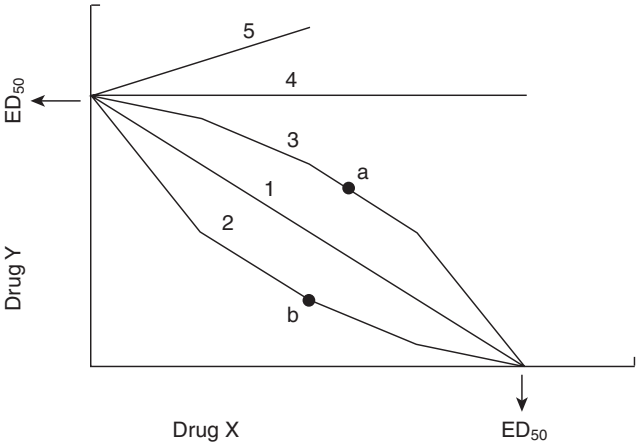

Figure 3-4. Graphical representation of isoboles for drugs "X" and "Y." Plot 1 is the line of additive effect (i.e., no or zero-interaction). The intercepts of that line represent the respective ED_{50} doses of the individual drugs that produce the chosen level of effect when administered alone. Plot 2 is the isobole for a supra-additive or synergistic interaction. Plot 3 is the isobole for an infra-additive interaction (i.e., antagonism). Plot 4 is the isobole when drug X is "inert." Plot 5 is the isobole when drug X is a competitive antagonist of drug Y. See text for discussion of points "a" and "b" on the isobologram.

of the doses of the drugs lie above and to the right of the line (i.e., plot 3) of additive effect, the interaction is termed *infra-additive* or *antagonism*. Also, points may appear on the graph when one of the drugs tested is "inert" (i.e., plot 4) or when one of the drugs (e.g., X) is a competitive receptor antagonist of the other drug (Y; plot 5).

In many instances, the stimulus effects of only a single combination of drugs might be tested, such that only a single point is plotted (e.g., point "a" or "b" in Figure 3-4). Whether these points represent a drug combination that is supra-additive, additive, or infra-additive is decided after a determination is made that the point lies sufficiently "far enough" below or above the line of additive effect. One method of determination involves the observation of whether or not the measured ED_{50} value for the combination falls within the area that is contained by connecting the 95% confidence intervals of the drugs alone [for details, see 139]. Examples of isobolographic analyses in drug discrimination studies can be found in investigations of interactions between cocaine and caffeine [140], pentobarbital and ethanol [141], chlordiazepoxide and triazolam [142], and two-drug combinations of various 5-HT agents [143]. Finally, *potentiation* is another type of interactive effect between drugs that occurs when the selected dose of one drug (i.e., test agent) is ineffective alone but when combined with another drug (i.e., training drug) produces a stimulus effect that is greater than the algebraic sum of their individual stimulus effects (not displayable on isobologram of Figure 3-4).

2. Comparison by Molecular Weights

When ED_{50} or AD_{50} values of agents that have substantially different molecular weights are employed to compare potency, those weight differences should be accounted for and reported on a micromole per kilogram (μmole/kg), rather than mg/kg, basis. This conversion takes into account the different molecular weights of the agents. For example, the molecular weight of amphetamine hydrochloride is approximately 171 (Table 3-3). The molecular weight of (the hypothetical) pentaiodoamphetamine hydrochloride is 806. Hence, if the latter agent had substituted in amphetamine-trained animals and was found equipotent on the basis of mg/kg, then (in reality) it would actually be about four times more potent than amphetamine when its molecular weight was taken into consideration.

Even different salts of the same agent have different molecular weights. For example, the molecular weight of free-base amphetamine is 135 whereas that of its

TABLE 3-3. A comparison of molecular weights of various forms of amphetamine

Amphetamine form	Molecular weight
Free base	135
Hydrochloride (HCl)	171
Hydrobromide (HBr)	216
Phosphate	233
Hydroiodide (HI)	263
Sulfate	354

phosphate salt is 233 (Table 3-3). The two drugs could be examined in tests of stimulus generalization in *S*(+)amphetamine-trained animals (assuming free-base amphetamine was water soluble). Hypothetically, amphetamine, assuming an ED_{50} value of 1.0 mg/kg, could appear twice as potent as amphetamine phosphate if the ED_{50} value of the latter was close to 2.0 mg/kg. However, a comparison of potencies on a μmole/kg basis would show that each agent possessed an ED_{50} value of ~7.5 μmoles/kg, which indicates that they are equipotent.

Consequently, potency differences of only 2- or 3-fold should be held suspect unless ED_{50} or AD_{50} values are reported on a μmole/kg basis. However, comparative data are usually reported on the basis of mg/kg. But, then again, conversions are only important when molecular weights differ substantially and where ED_{50} or AD_{50} values differ by several-fold. Doses in mg/kg can be converted to doses in μmole/kg by the following formula: moles = g/mol.wt.

3. Percentage Drug Lever Responding

In the examples of drug discrimination data presented here and in Chapters 4 to 7, the quantitative (or graded) approach was employed to analyze results; that is, calculation of percent drug-appropriate lever responding. An animal's degree of progress to learn a two-lever drug discrimination task was determined by an evaluation of its distribution of presses on the levers either prior to, or up to, the delivery of the first reinforcement. Thus, when the FR 10 schedule of reinforcement was in effect, discrimination learning was assessed for each subject by dividing the number of responses that occurred on the drug-designated lever by the total number of responses that occurred on both levers up to the delivery of the first reinforcement; percent drug-appropriate lever responding was then obtained by multiplication of that value by 100. For instance, a rat may have the right-side lever designated as the diazepam-appropriate lever. On a Monday, the animal is injected with 3.0 mg/kg of diazepam (15 minute PSII), placed in its assigned operant chamber, and proceeds to press on the left-lever 9 times and the right-lever 10 times; food reinforcement (in this example) would be presented after the 10th right-side lever press. For this day, discriminative control would be assessed at 53% diazepam-appropriate responding (i.e., 10 right-side lever presses divided by 19 total lever presses × 100). On Tuesday, that same rat is injected with vehicle (15 minute PSII), placed into its designated chamber, and presses the right-lever 4 times and the left-lever 10 times; food is presented after the 10th left-side press. On this day, discriminative control would be assessed at 29% diazepam-appropriate responding (i.e., 4 divided by 14 × 100). If the group of animals had been trained under a variable interval (VI) schedule of reinforcement, then discrimination performance would have been evaluated during a short period (e.g., 2.5 minutes) of nonreinforced responding (referred to as extinction) at the beginning of a session followed by 12.5 minutes of responding that was reinforced; extinction sessions usually occur once or twice per week. Each animal's distribution of presses on the two levers would then be evaluated in the same manner as initial presses of levers under the FR schedule of reinforcement as described above. In either situation, incorrect responses did not have programmed consequences.

During initial training sessions, as might be expected, subjects typically divide their responses equally between the two levers after administration of dose of training drug or vehicle under either FR or VI schedules of reinforcement. However, animals gradually learn, as training sessions with the treatments progress, to respond on the drug-designated lever (i.e., percent of responses on the drug–designated lever is *high* and the number of responses on the vehicle-designated lever is *low*) when administered their dose of training drug, and to respond on the vehicle-designated lever (i.e., percent of responses on the drug–designated lever is *low* and the number of responses on the vehicle-designated lever is *high*) when given vehicle. In most cases, drug discriminations are learned gradually. For example, Figure 3-5 demonstrates a learning curve for a diazepam *versus* vehicle discrimination (top figure) under an FR 10 schedule of

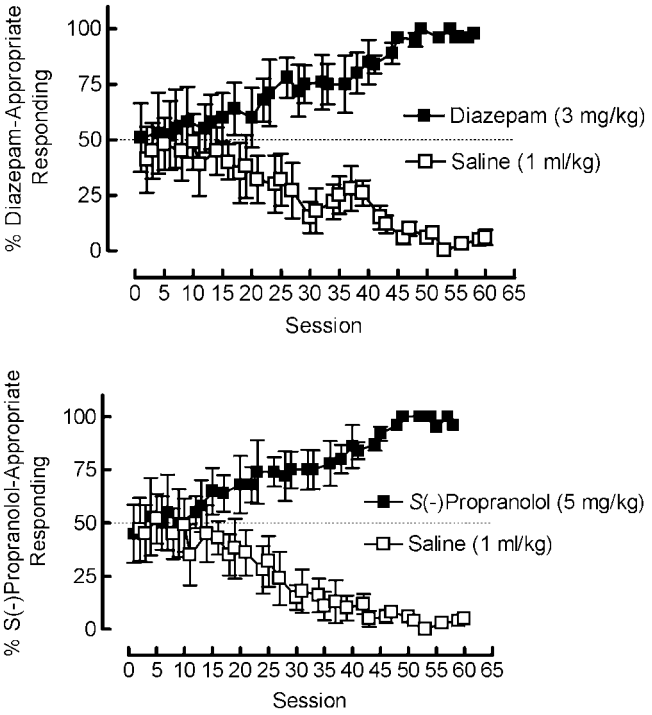

Figure 3-5. Learning curves of rats trained to discriminate the stimulus effects of either 3.0 mg/kg of diazepam (top graph, closed squares) or 5.0 mg/kg of S(–)propranolol (bottom graph, closed squares) versus 1.0 ml/kg of vehicle (both graphs, open squares). Ordinate: Mean percent (± S.E.M.) of responding on the diazepam- or S(–)propranolol-appropriate lever after the administration of drug or vehicle. Abscissa: Number of sessions for the animals to learn to respond on the diazepam- or S(–)propranolol-designated lever when administered drug (i.e., percent of responding is high on the drug-assigned lever and number of responses is low on the vehicle-designated lever) and on the vehicle-designated lever when administered vehicle (i.e., percent of responding is low on the diazepam- or S(–)propranolol-assigned lever and number of responses is high on the vehicle-designated lever).

reinforcement and a *S*(−)propranolol *versus* vehicle discrimination (bottom figure) under a VI 15-second schedule of reinforcement.

A general guideline is that after 6 to 9 weeks of training, animals (individually and, consequently, as a group mean) consistently make ≥80% of their responses on the drug-appropriate lever after administration of dose of drug and ≤20% of their responses on the same lever after administration of vehicle. *Importantly, subjects' learning of drug discriminations is defined by the* separation *between their percentage drug-appropriate responding after administration of dose of training drug versus their percentage drug-appropriate responding after administration of vehicle* (see sessions 40–60 for each training drug in Figure 3-5). As mentioned previously (Section B, above), some discriminations are acquired quickly but others are learned after changes in methodology and/or numerous training sessions. Glennon and Young [49], for example, reported that rats required 6 to 8 months to learn the discriminative stimulus effects of 1.5 mg/kg of (±)MDA (1-(3,4-methylenedioxyphenyl)-2-aminopropane) *versus* vehicle. On the other hand, only 2 to 3 weeks were required to train rats to discriminate the stimulus effects of 5.0 mg/kg of TDIQ (5,6,7,8-tetrahydro-1,3-dioxolo[4,5-*g*]isoquinoline) *versus* vehicle [144]. Figure 3-6 shows that the rats demonstrated substantial separation in their %TDIQ drug-appropriate responding within 2 weeks of training and met the criteria for drug discrimination (see above) by the third week of training. Once achieved, the animals maintained the discrimination for ~2 years.

4. Response Rate

In addition to the animals' distribution of responses on the two levers under FR or VI schedules of reinforcement, their response rate data (i.e., total number of responses on

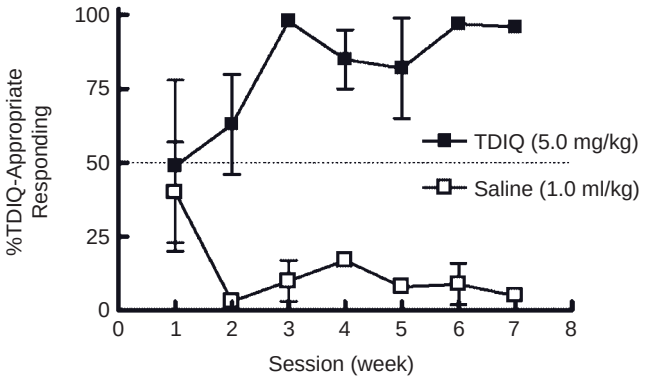

Figure 3-6. Discrimination learning curve for rats trained to distinguish TDIQ versus saline vehicle. Ordinate: Mean percent (± S.E.M.) of responses performed on the TDIQ-designated lever after the administration of 5.0 mg/kg of TDIQ (solid squares) versus 1.0 ml/kg of 0.9% saline (open squares). Abscissa: Each number represents a pair of test sessions conducted during a week (total of 7 weeks of training).

both levers expressed as responses per second or minute) also can be calculated and presented. For example, animals' (individual and/or group mean) response rate can be measured under the FR schedule of reinforcement for a behavioral session (e.g., responses per second or minute over the duration of the session). Under the VI schedule of reinforcement, response rates performed during the 2.5 minute extinction sessions (and/or the entire session) can be recorded. The animals' response rate can be viewed as another indicator of the effects of a drug on behavior. In many instances, animals' response rate after the dose of the training drug is comparable (i.e., not statistically different) to that after vehicle but in some cases is somewhat suppressed when compared to that of vehicle. Also, response rate data can assist the experimenter in the selection of 1) an appropriate training dose of the training drug and/or 2) a range of doses to be examined for test drugs. Further, animals' response rate can be an ancillary measure in cases where a test drug may, or may not, affect the main dependent variable (i.e., percent responding on the drug-designated lever). For example, even though a quaternary amine analog may not markedly affect discrimination results (i.e., percent drug-appropriate responding), an organism's response rate may be affected (see Section E, 2a below). As such, discriminative stimulus effects can occur or be independent from response rate. Lastly, response rates can be used in conjunction with an evaluation of the subjects' general behavioral condition (e.g., sedated, incapacitated, hyperactive). In the present review (Chapters 3 to 7), however, an emphasis is placed on discrimination results (i.e., percent drug-appropriate responding) unless there is an overriding reason to comment on response rates.

E. TESTING

1. Stimulus Generalization (Substitution)

Stimulus generalization refers to an event in which a response (i.e., drug-designated lever responding) that has been reinforced in the presence of a given dose of a training drug occurs with an increased frequency in the presence of doses of a test agent either prior to, up to, or without (i.e., under nonreinforced extinction conditions) the presentation of reinforcement. A generally accepted standard is that stimulus generalization of the dose of the training drug is considered to have occurred (i.e., is complete) to an administered dose of test agent if subjects respond to a high degree on the drug-designated lever (e.g., group mean of ≥80% of responses; ideally, each member of the group produces ≥80% drug-appropriate responding). It is noted, however, that the phrase "stimulus generalization of the vehicle to the test agent" is not used when the animals respond on the vehicle-designated lever after administration of the test treatment; such results are characterized as the dose of test agent produced "responding on the vehicle–designated lever" or simply "vehicle-like responding or a vehicle-like response." In the present examples (see Section C above), maintenance of discriminations between each dose of training drug *versus* vehicle was ensured by continuation of training sessions that were interspersed between stimulus generalization test ses-

sions. Training sessions were conducted in each group with the dose of training drug or vehicle on the four days prior to a stimulus generalization test session. On at least one of those days, half of the animals received the dose of training drug and the other half received vehicle; percent drug-appropriate responding was then determined under either the FR or VI schedule of reinforcement as described above (see Section D, #3). If an animal did not meet the above criteria (i.e., ≥80% drug-appropriate responding after drug administration of the training dose of training drug, and ≤20% drug-appropriate responding after vehicle injection), then it was not used in that week's stimulus generalization test. In such tests, the rats were administered a dose of test agent and then allowed to press on the lever(s) in a session that lasted 10 min (e.g., FR 10 procedure). The lever on which the animal performed 10 presses was regarded as the selected lever and the rat was removed from the apparatus; percent drug-appropriate lever responding was calculated as described above (Section D, #3). Some investigators, however, will present reinforcement after the 10th press on a selected lever and may, in fact, allow (if time permits) the animal to continue to respond on the selected lever according to the FR 10 schedule of reinforcement. If a group of rats had been trained under the VI schedule of reinforcement, then they would have been administered a dose of test agent, given an extinction session (e.g., nonreinforced responding for 2.5 minutes) and removed from the operant chambers; percent drug-appropriate lever responding would have been calculated as described above. *Under any schedule of reinforcement, the degree of stimulus generalization produced by doses of test agents is assessed only with those responses that occur prior to, up to, or without the presentation of reinforcement*. The latter statement is important because once a subject is reinforced in any session, the presentation of reinforcement on subsequent responding may become as important, or more so, than the stimulus generalization effects of the dose of test drug. Lastly, results of stimulus generalization tests with a particular dose of a training drug have typically been consistent across species (see Table 2-1) and schedules of reinforcement (see Table 2-3).

2. Issues

Tests of stimulus generalization (or stimulus antagonism; see below) with a training drug or test agent should be conducted with a thorough dose-response evaluation; single dose studies of a drug are rarely, if ever, sufficient. The dose-response effect, function, or curve refers to the observation that, as the amount of a drug that is administered to subjects is varied, there may be a change in their percent drug-appropriate responses. Notably, a dose-response is obtained from subjects in whom stimulus control is already established at a given dose of the training drug (and PSII); tests are then conducted with other doses of the same drug or with another agent. Dose-response data are typically graphed as semi-logarithmic plots: percent drug-appropriate responses plotted on a linear scale on the ordinate (y-axis) and doses plotted on a $\log_{10}$ scale on the abscissa (x-axis). The latter transformation, in comparison to dose-response data where doses also are plotted on a linear scale, will often yield a sigmoid or S-shaped curve with a central portion of the dose-response that is approximately linear.

a. Central versus Peripheral Sites of Action Agents typically interact with neurotransmitter receptors in the peripheral nervous system and, if the drug is suitably lipophilic (i.e., lipid-soluble) and crosses the blood brain barrier (BBB) in the CNS. If it does not penetrate the BBB, then it will not be psychoactive. Studies of drugs as discriminative stimuli, as well as other procedures that study the interactions of drugs and behavior, however, are often predicated on the assumption that if an agent affects an organism's behavior, then the changes were mediated primarily, if not exclusively, by the actions of the drug in the CNS. Clearly, the administration of drugs can exert effects on behavior via sites of action that are apart or aside from the CNS. For example, agents might affect behavioral activity via an action at the neuromuscular junction, the autonomic nervous system, or the site of (an accidentally inferior) injection. Thus, an important part of the characterization of the mechanism of a drug-induced change in behavior is the assessment of the relative contribution of peripheral versus central sites of action. Studies of the stimulus properties of drugs have employed a number of approaches to gauge the relative contribution of peripheral versus central effects of an agent. In one approach, drugs are administered centrally (e.g., intracerebrally or intraventricularly) *versus* parenterally (e.g., subcutaneously or intraperitoneally) and compared for effect. For example, Schechter [145] trained one group of rats to discriminate the subcutaneous (s.c.) administration of 0.4 mg/kg of (–)nicotine *versus* vehicle and a second group to discriminate the intraventricular administration of 644 ng of (–)nicotine *versus* vehicle. In tests of stimulus generalization, rats trained to (–)nicotine by the subcutaneous route generalized (substituted) to (–)nicotine administered intraventricularly and rats trained by the intraventricular route generalized to (–)nicotine given subcutaneously. Similar results have been reported in other studies, some of which include the injection of drugs to localized sites in the brain, with (–)nicotine [146–150], mescaline [151], (+)lysergic acid diethylamide [152], thyrotropin-releasing hormone [153], morphine [154–160], phencyclidine [161], scopolamine [162], cocaine [163, 164], midazolam [165], and (±)cathinone [166]. The studies provide evidence, at least in part, for a CNS locus of action for those drugs.

Another approach that is employed to differentiate peripheral *versus* central action of a drug is to limit or block BBB transport and, thus, highlight selective effects in the periphery. For example, passage of a (parent) drug through the BBB can be restricted or prevented by the addition of polar functional groups such that the peripheral but not the central actions of the agents are similar. The approach can be evaluated by a comparison of the dose-response curves for the presumed CNS effect of the two similar compounds: one (parent) that enters readily and one (polar analog) that passes through with some degree of difficulty (or not at all) into the brain. If the discriminative stimulus effect of a drug is mediated primarily by peripheral action, then the dose response curves of the two drugs should be approximately equipotent. For example, the BBB transport of a parent drug can be hindered by the addition of a hydroxyl group, which can result in an agent that is relatively more polar and less likely to penetrate the barrier. The "hydroxyl strategy" has been tested numerous times with dose-effect data obtained from animals trained to discriminate amphetamine from vehicle and then tested with para-hydroxyamphetamine (a.k.a., 4-hydroxyamphetamine; see structure in Chapter 4, Figure 4-4), which is unlikely to readily penetrate the BBB and exert central activity

because of the presence of its polar hydroxyl group but mimics the peripheral effects of amphetamine. In animals (rats, mice, gerbils, and pigeons) trained to discriminate various doses (from 0.8 to 8.0 mg/kg) of *S*(+)amphetamine *versus* vehicle, results from stimulus generalization tests are very consistent that 4-hydroxyamphetamine is not recognized as being amphetamine-like [167–171]. The results suggest that the stimulus effects of those doses of *S*(+)amphetamine are centrally mediated.

A third approach involves the chemical class of amines, which are organic compounds and a type of functional group that contain nitrogen as the key atom. In structure, amines resemble ammonia, wherein one or more hydrogen atoms are replaced by an organic substituent such as alkyl or aryl groups. When applied to amines, the terms primary, secondary, tertiary, or quaternary (e.g., Figure 3-7) refer to the number of alkyl (or aryl) substituents bonded to the nitrogen atom. The amines are electrically neutral except for the quaternary amine, which bears a permanent positive charge that can minimize or prevent passage of the molecule across a lipophilic membrane like the BBB. As a class of agents, quaternary amines exhibit hydrophilic character with low partition coefficients, which makes it difficult for them to cross lipid cell membranes. Thus, quaternary amines are poorly absorbed from the gastrointestinal tract, have low bioavailability, and have poor (but perhaps not totally incomplete) penetration into the CNS. Consequently, they are often referred to as *peripherally acting* because of their inability to readily traverse the BBB of the CNS.

In one early study, Rosecrans and Chance [172] demonstrated that the discriminative stimulus effects of (–)nicotine were antagonized by mecamylamine, a voltage dependent noncompetitive channel blocker at nicotinic receptors that penetrates the BBB, but were not antagonized by hexamethonium, a quaternary ammonium ganglionic blocker that does not readily cross the BBB. Other studies have employed the "quaternary amine approach" with animals trained to discriminate, or tested with, various (parent) agents and also tested with quaternary amine analogs. For example, Glennon and colleagues [117] trained rats to discriminate 5.0 mg/kg of the purported 5-HT_3 receptor agonist 2-methylserotonin (2-Me 5-HT) *versus* vehicle. The 5-HT_3 receptor antagonist tropisetron (a.k.a., ICS 205–930) potently antagonized the 2-Me 5-HT stimulus (AD_{50} = 0.001 mg/kg), whereas its quaternary amine analog (i.e., tropisetron methiodide), which does not readily penetrate the BBB, failed to completely antagonize the 2-Me 5-HT stimulus at a 10,000-fold higher dose (Figure 3-8). The results strongly suggest that the stimulus effects of 2-Me 5-HT are likely mediated via a central 5-HT_3 mechanism.

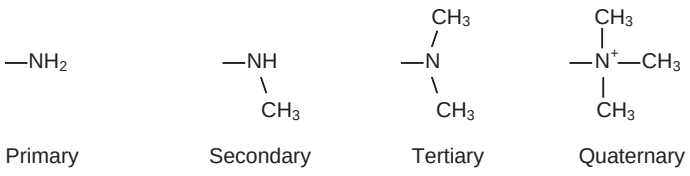

Figure 3-7. Structural representation of primary through quaternary amines.

Table 3-4 lists drug stimuli, test drugs, quaternary amine analogs, discrimination results, and response rate characterizations from several studies. Drugs that have been used as training stimuli and their quaternary amine counterparts include nicotine (nicotine methiodide hydroiodide), cocaine (cocaine methiodide), *R*(−)DOB (*N*,*N*,*N*-trimethyl-DOB iodide), atropine (methylatropine), naloxone (naloxone methobromide), and naltrexone (naltrexone methobromide). In other studies, tests of stimulus generalization or antagonism have used agents such as tropisetron and its quaternary amine tropisetron methiodide, nalorphine (*N*-methyl nalorphinium bromide), and scopolamine (methylscopolamine). The drug discrimination results in Table 3-4 show that in each study the parent agent resulted in either stimulus generalization or antagonism, but its quaternary amine analog produced neither of these results (see column labeled Discrimination Result). It should be noted, however, that not all of the quaternary amines exhibit similar binding affinities as their parent agents. For example, *N*,*N*,*N*-trimethyl-DOB iodide does not bind at 5-HT_2 receptors and, as such, even if it was to enter the CNS, it might still not produce *R*(−)DOB-like stimulus effects [26]. Nonetheless, the vast majority of these studies provide very strong, if not the strongest, evidence for the conclusion that, when tested under comparable conditions, agents that readily enter the CNS appear to be much more efficient as training drugs and test agents than those that do not. This does not imply, however, that an agent that acts peripherally cannot affect behavior. Thus, even though a quaternary amine analog may not affect

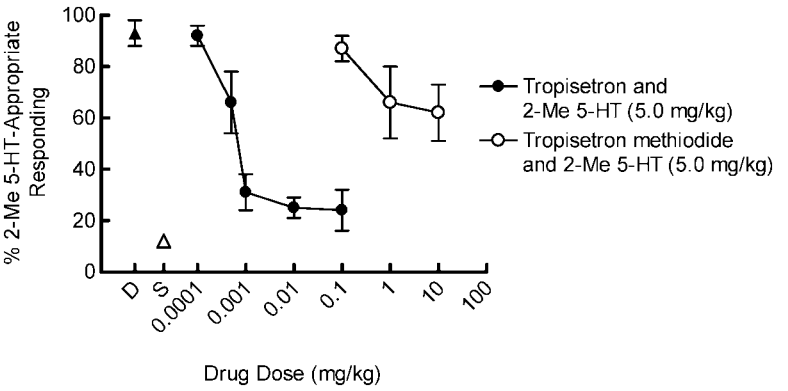

Figure 3-8. The effects of doses of tropisetron (closed circles) or tropisetron methiodide (open circles) administered in combination with 5.0 mg/kg of 2-Me 5-HT in rats trained to discriminate 5.0 mg/kg of 2-Me 5-HT versus vehicle. Ordinate: Mean percent (± S.E.M.) 2-Me 5-HT-appropriate lever responding. The administration of tropisetron prior to the injection of 5.0 mg/kg of 2-Me 5-HT produced dose related antagonism (AD_{50} = 0.001 mg/kg) of the stimulus effect of 2-Me 5-HT. In comparison, the administration of tropisetron methiodide before the injection of 5.0 mg/kg of 2-Me 5-HT failed to completely antagonize the 2-Me 5-HT stimulus at a 10,000-fold higher dose (i.e., 10 mg/kg). D and S represent percent 2-Me 5-HT-appropriate responding following the training dose of 2-Me 5-HT or saline, respectively.

TABLE 3-4. Summary of drug discrimination studies with animals trained to discriminate, or tested with, various (parent) agents and also tested with quaternary amine analogs. Table includes training drug, parent agent and its quaternary amine analog, discrimination results, and effect on response rate

Training Drug	Test Agent	Discrimination Result[a]	Response Rate	Reference
Arecoline	Atropine	A	*	Schechter & Rosecrans [4]
	Methylatropine	NA	*	
Atropine	Atropine	G	**	Kubena & Barry [32]
	Methylatropine	NG	**	
Clozapine	Scopolamine	G	↓	Nielsen [178]
	Methylscopolamine	NG	↔	
Cocaine	Cocaine	G	↔	Witkin et al. [179]
	Cocaine methiodide	NG	↔	
Cocaine	Cocaine	G	↔	Terry et al. [173]
	Cocaine methiodide	NG	↓	
R(−)DOB[b]	*R*(−)DOB	G	↔	Glennon et al. [26]
	N,*N*,*N*-trimethyl-DOB iodide	NG	↓	
Ethylketazocine	Nalorphine	G	↔	Hein et al. [33]
	N-methyl nalorphinium bromide	NG	↔	
Ethylketazocine	Naltrexone	A	↔	Hein et al. [33]
	Naltrexone methobromide	NA	↓	
Etorphine	Naltrexone	A	↑	Valentino et al. [180]
	Naltrexone methobromide	NA	↔	
2-Methylserotonin	Tropisetron	A	↔	Glennon et al. [117]
	Tropisetron methiodide	NA	↔	
Morphine	Naltrexone	A	↓	Valentino et al. [180]
	Naltrexone methobromide	NA	↓	
Morphine	Naltrexone	A	****	Locke & Holtzman [181]
	Naltrexone methobromide	NA		
Naloxone	Naloxone	G	***	Medvedev et al. [182]
	Methylnaloxone	NG	***	
Naltrexone	Naltrexone	G	↓	Valentino et al. [180]
	Naltrexone methobromide	NG	↓	
(−)Nicotine	Nicotine	G	*	Schechter & Rosecrans [62]
	Nicotine methiodide hydroiodide	NG	*	

[a]A = Antagonism; NA = No Antagonism; G = Generalization; NG = No Generalization.

[b]*R*(−)-1-(4-Bromo-2,5-dimethoxyphenyl)-2-aminopropane.

*T-maze study—response rate not applicable.

**Response rate data not reported.

***Y-Maze study—response rate not applicable.

****Discrete-trial avoidance task—response rate not applicable.

↔ No change from vehicle control; ↓ Marked decrease from vehicle control; ↑ Marked increase from vehicle control.

discrimination results (defined as percent drug-appropriate responding), an organism's response rate may be affected. For example, Terry et al. [173] trained rats to discriminate the effect of cocaine from vehicle and demonstrated that the stimulus generalized in a dose-related manner to cocaine but not to the quaternary amine cocaine methiodide. The latter agent did, however, produce a dose-related reduction in the animals' response rates, which suggests that the peripheral action of the quaternary amine drug affected behavior; other quaternary analogs (e.g., naltrexone methobromide) also have shown such an effect on *response rates*, whereas others (e.g., *N*-methyl nalorphinium bromide, tropisetron methiodide) have not [33, 117]. In addition, the assumption should not be made that an agent that acts peripherally, or that a peripheral effect of an agent that also acts in the CNS is unable to serve as a discriminative stimulus. On the contrary, Colpaert et al. [44] reported that isopropamide, a quaternary amine anticholinergic drug, can serve as a discriminative stimulus in rats. Also, Colpaert et al. [174] and White and Appel [175] have reported that low doses of (±)amphetamine and (+)LSD, respectively, can serve as stimuli that are (primarily) peripherally mediated in rats. Lastly, Schuh et al. [176] reported that the stimulus effects of cocaine, administered intranasally to humans, are likely mediated by a peripheral mechanism.

In summary, CNS-active drugs (i.e., those that penetrate the BBB) appear to be more effective as discriminative stimuli than agents that act peripherally. However, agents that act peripherally can affect behavior (e.g., response rates) and, in fact, have been shown in some instances to function as discriminative stimuli. In this regard, it is curious (albeit understandable) that drugs that exert their effects primarily in the peripheral nervous system have not seen anywhere near the degree of effort expended on their potential as discriminative stimuli as have drugs that exert their effects primarily in the CNS. A speculation to possibly explain this state of affairs may be that the "motivation conditions," such as restriction of food/water or escape/avoidance of shock, used most often in drug discrimination studies are mediated (primarily) by central mechanisms and, therefore, those conditions are possibly more "conducive" for a subject to learn the effects of a drug in the CNS. If so, perhaps discrimination methods and procedures that place more emphasis on "peripheral conditions" might be more favorable to the establishment of peripheral agents as stimuli. For example, Weissman [177] demonstrated that acetylsalicylic acid (a.k.a., 2-acetoxybenzoic acid, aspirin) is discriminated much better in rats made arthritic, through injection of *Mycobacterium butyricum* into a hind paw, than in rats that were nonarthritic. In any case, investigations of the potential stimulus properties of drugs that exert their effects primarily in the peripheral nervous system might benefit from the introduction of innovative methodologies.

b. Complete versus Incomplete Generalization *The term "stimulus generalization" should be reserved for the actual demonstration that two agents can exert a similar stimulus effect*. In subjects trained to discriminate a dose of drug from vehicle, a prudent practice is to evaluate doses of a test agent until the occurrence of either stimulus generalization (e.g., group mean of ≥80% drug-appropriate responding; ideally, each member of the group produces ≥80% drug-appropriate responding) or disruption

of behavior (i.e., <50% of the subjects respond or little/no responding occurs). On the other hand, if the highest dose (e.g., dose X) of a test drug resulted in ~50% drug-appropriate responding, and, for some reason, the evaluation of higher doses is precluded, there are a number of practices that should be avoided such as: (a) concluding that the test drug is half a potent as the training drug, (b) claiming dose X as an ED_{50} value, or (c) applying regression analysis on the "incomplete" data to extrapolate and "complete" the upper segment of the dose-effect function and then calculating an ED_{50} value (or AD_{50} value in an antagonism test). In such a case, comparisons should only be determined on a qualitative basis. That is, an appropriate conclusion might be that the challenge agent is less effective than the training drug to produce a training-drug-like effect (or, correspondingly, that it is less effective than some other challenge drug which, at a dose below dose X, produced training-drug-like effects). Likewise, if two challenge drugs produce intermediate percentage drug-appropriate responding (e.g., 40% and 60% training-drug-appropriate responding respectively) at dose X, it should not be stated with certainty that the second challenge drug is more potent than the first because the possibility exists that one (or both) agent(s) may not exert a stimulus effect that is similar (i.e., may not generalize fully) to that of the training drug (see discussion of partial generalization below).

c. Dose Response Functions Are Relative *The results of any dose-response test that is performed in a drug discrimination study are always analyzed in relation to the dose and PSII of the training drug that is administered in training sessions and should not be viewed as being absolute*. For example, the dose-response curve that is obtained from animals trained on a relatively high dose of a drug at a particular PSII will occur to the right of the dose response curve that is obtained in a second group of animals that is trained on a relatively low dose but same PSII of that same drug. In other words, when separate groups of subjects are trained with markedly different doses of the same training drug, they will generate markedly different dose-response curves; that is, subjects' training history is of utmost importance [e.g., 127 (data described in Section D #1); 175]. Consequently, dose-response functions obtained in drug discrimination studies are relative to the dose of training drug and comparisons to dose-response effects of that drug in other behavioral procedures are not appropriate.

d. Stimulus Effects Are Relative *Results of stimulus generalization tests are interpreted in relation to the dose of training drug-like effects*. For example, an investigation of the effects of barbiturate analogs in diazepam-trained animals does not provide data on barbiturate-like activity. Such data reflect the actions of those barbiturate analogs to produce "diazepam-like" stimulus effects. Similarly, a study of benzodiazepine analogs in pentobarbital-trained animals does not provide data on benzodiazepine-like action but reflect the actions of those analogs to produce "pentobarbital-like" stimulus effects. In many instances, agents will exhibit *cross-generalization* such that stimulus generalization occurs between agents regardless of which drug was employed as the training stimulus. Figure 3-9 shows the dose related cross-generalization of benzodiazepine and barbiturate analogs in rats trained to dis-

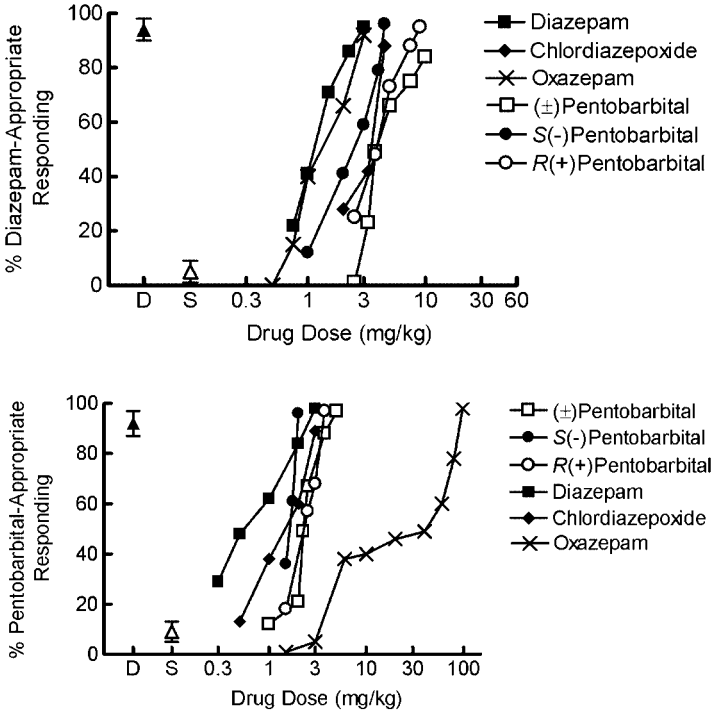

Figure 3-9. Dose-response relationships for benzodiazepine and barbiturate analogs in rats trained to discriminate either 3.0 mg/kg of diazepam (top figure) or 5.0 mg/kg of pentobarbital (bottom figure) versus vehicle. Ordinate: Data points represent group means (± S.E.M. not shown for purpose of clarity) of percent diazepam- or percent pentobarbital-appropriate lever responding after the administration of the test agents in each group of subjects. Abscissa: Drug dose plotted on a logarithmic scale. D represents percent responding following the training doses of 3.0 mg/kg of diazepam or 5.0 mg/kg of pentobarbital; S represents percent responding following administration of saline vehicle. Typically, response rates for each group would be presented in a separate figure situated below each % drug-appropriate responding figure.

criminate either 3.0 mg/kg of diazepam (top figure) or 5.0 mg/kg of pentobarbital (bottom figure) *versus* vehicle.

Table 3-5 presents a summary of ED_{50} values (in mg/kg and μmole/kg) of the agents in each group of rats. In subjects trained to discriminate diazepam from vehicle, the order of potency of the agents (based on μmole/kg) is diazepam ≥ oxazepam > *S*(−) pentobarbital ≥ chlordiazepoxide > *R*(+)pentobarbital ≥ (±)pentobarbital. In comparison, in rats trained to discriminate pentobarbital from vehicle, the order of potency of the agents is diazepam > chlodiazepoxide > *S*(−)pentobarbital > (±)pentobarbi-

TABLE 3-5. Summary of ED_{50} values of benzodiazepine and barbiturate analogs in rats trained to discriminate either diazepam (3 mg/kg) or pentobarbital (5 mg/kg) from vehicle; dose response data shown in Figure 3-9

	Diazepam Stimulus ED_{50} Values[b]		(±)Pentobarbital Stimulus ED_{50} Values[b]	
Agent[a]	mg/kg	μmole/kg	mg/kg	μmole/kg
Diazepam (285)	1.22	4.3	0.55	1.9
Chlordiazepoxide HCl (336)	3.43	10.2	1.31	3.9
Oxazepam (287)	1.35	4.7	55.58	193.7
(±)Pentobarbital Na (248)	4.44	17.9	2.27	9.2
S(−)Pentobarbital (225)	2.20	9.8	1.58	7.0
R(+)Pentobarbital (225)	3.86	17.2	2.20	9.8

[a]Molecular weight of compound in parenthesis.
[b]ED_{50} values expressed in mg/kg and in equivalent μmole/kg.

tal ≥ *R*(+)pentobarbital > oxazepam. Within each group of subjects, the potency relationships between the agents are relatively similar with one glaring exception: oxazepam, which is almost equipotent to diazepam and approximately four times more potent than (±)pentobarbital in the diazepam-trained animals, but is almost 100 times less potent than diazepam and more than 20 times less potent than (±)pentobarbital in the pentobarbital-trained subjects. These results re-emphasize the idea that stimulus generalization results occur and are interpreted in relation to the dose of training drug-like effects after a thorough dose-response evaluation.

In other instances, investigators have reported cases of "asymmetric (or one-way) stimulus generalization," which occurs when subjects (trained to a dose of drug) exhibit stimulus generalization to a test agent but when a separate group of subjects is trained to that test agent as training drug they do not exhibit stimulus generalization to the other drug. *Asymmetric stimulus generalizations* have been reported between (−) nicotine and cocaine [183], ethanol and NMDA (*N*-methyl-D-aspartate) receptor antagonists [184], (±)MDMA (*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane) and cocaine [119, 185], and (−)ephedrine and *S*(+)methamphetamine [186]. For example, Young and Glennon [187] and Bondareva et al. [186] trained rats to discriminate the stimulus effects of either 4.0 mg/kg of (−)ephedrine or 1.0 mg/kg of *S*(+) amphetamine *versus* saline vehicle. The (−)ephedrine stimulus (ED_{50} = 0.8 mg/kg) generalized to various central stimulants including *R*(+)methamphetamine (ED_{50} = 0.9) but failed to generalize to *S*(+)methamphetamine after a thorough dose response evaluation (Figure 3-10). In comparison (Figure 3-11), the *S*(+)methamphetamine stimulus (ED_{50} = 0.06 mg/kg) also generalized to various central stimulants including *R*(+)methamphetamine (ED_{50} = 1.61 mg/kg) and (−)ephedrine (ED_{50} = 13.1 mg/kg). Thus, *S*(+) methamphetamine-trained animals generalized to (−)ephedrine but (−)ephedrine-trained animals failed to generalize to (i.e., recognize) *S*(+)methamphetamine.

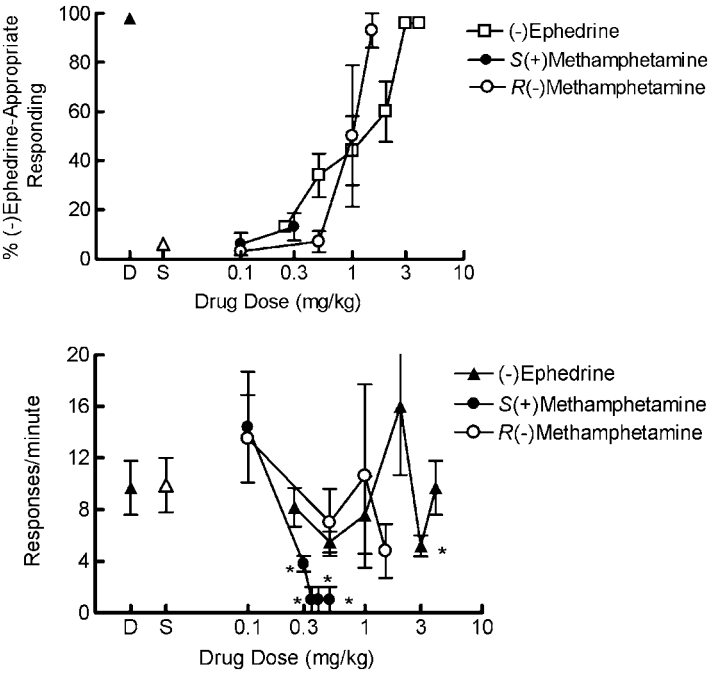

Figure 3-10. Mean (± S.E.M.) % (–)ephedrine-appropriate responding (top figure) and response rate data (bottom figure) performed by rats trained to discriminate 4.0 mg/kg of (–) ephedrine versus vehicle following the administration of various doses of (–)ephedrine (solid triangles), R(–)methamphetamine (open circles), and S(+)methamphetamine (closed circles). The (–)ephedrine stimulus failed to generalize to S(+)methamphetamine; doses of ≤0.3 mg/kg produced a maximum of 13% (–)ephedrine-appropriate responding (top figure) and ≥0.3 mg/kg of S(+)methamphetamine produced severe disruption of response rates (close to or no responding; bottom figure). D and S represent percent (–)ephedrine-appropriate responding (top figure) or responses/minute (bottom figure) following the training dose of (-ephedrine) or saline, respectively.

The occurrence of asymmetric stimulus generalization might be related, at least in part, to differences in the extent to which each agent interacts with neurotransmitter receptor mechanisms, and to which of these mechanisms predominates in the respective stimulus effects of the dose of training drug and/or test agent [e.g., see discussions in 185–188]. *Thus, the more (or less) the pharmacological features of a dose of test agent resembles the stimulus conditions present during training, the greater (or smaller) the probability that the trained response (i.e., drug-appropriate responding) will be emitted, respectively.*

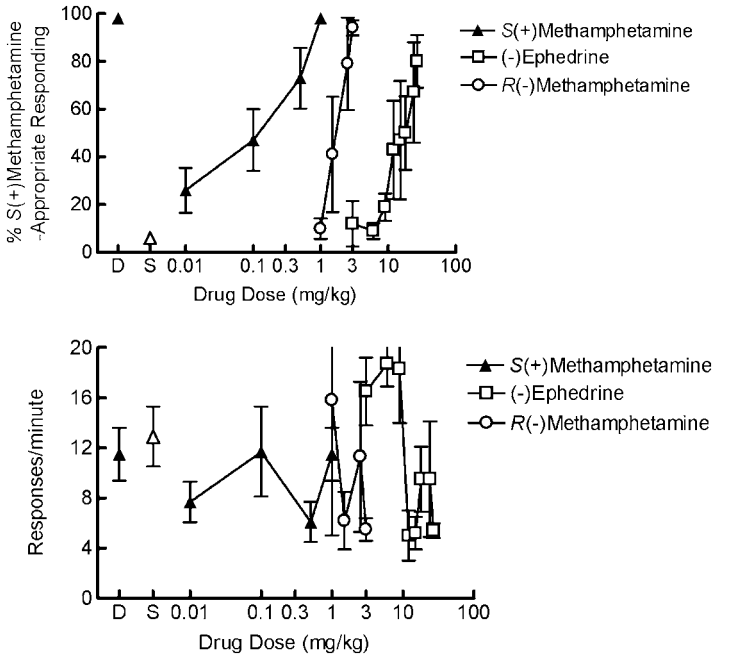

Figure 3-11. Mean (± S.E.M.) % S(+)methamphetamine-appropriate responding (top figure) and response rate data (bottom figure) performed by rats trained to discriminate 1.0 mg/kg of S(+)methamphetamine versus vehicle following the administration of various doses of S(+)methamphetamine (ED_{50} = 0.06 mg/kg; closed triangles), (–)ephedrine (ED_{50} = 13.1 mg/kg; open squares), or R(–)methamphetamine (ED_{50} = 1.61 mg/kg; open circles). D and S represent percent S(+)methamphetamine-appropriate responding (top figure) or responses/minute (bottom figure) following the training dose of S(+)methamphetamine or saline, respectively.

e. Dose-Dependent (Training Dose) Stimulus Effects *A critically important factor in data interpretation is that qualitatively or mechanistically different discriminative stimulus effects may be produced by different doses of the same drug*. Few drugs exert only one pharmacological effect. An agent selected for training in a drug discrimination experiment cannot be characterized completely by a single dose for study because different stimulus effects may be observed at different doses (e.g., see Chapter 1 with 5-OMe DMT as training drug). Table 3-6 lists studies that employed separate groups of subjects trained to discriminate different doses of the same drug and whether differences were noted in qualitative/mechanistic results or characteristics. As can be seen, most of the studies reported that different doses of the same drug can produce, to some degree, dissimilar stimulus effects. For example, rats trained to discriminate the effects of 0.3 mg/kg, but not 10 mg/kg, of morphine generalized

TABLE 3-6. List of studies that employed separate groups of subjects trained to discriminate different doses of the same drug from vehicle and whether a difference in qualitative and/or mechanistic results was noted

Training Drug	Training Dose Groups	Difference(s)	Reference
S(+)Amphetamine	0.4 mg/kg *vs.* vehicle 1.0 mg/kg *vs.* vehicle 1.6 mg/kg *vs.* vehicle	Yes	Stolerman & D'Mello [189]
β-CCE[a]	5.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Rowlett et al. [190]
Bremazocine	0.056 mg/kg *vs.* vehicle 0.17 mg/kg *vs.* vehicle	Yes	Smith & Picker [191]
Caffeine	10 mg/kg *vs.* vehicle 56 mg/kg *vs.* vehicle	Yes	Mumford & Holtzman [192]
Chlordiazepoxide	3.0 mg/kg vs. vehicle 15 mg/kg *vs.* vehicle	Yes	De Vry & Slangen [193]
Chlordiazepoxide	5.0 mg/kg *vs.* vehicle 20 mg/kg *vs.* vehicle	No	De Vry & Slangen [194]
Clozapine	1.25 mg/kg *vs.* vehicle 5.0 mg/kg *vs.* vehicle	Yes	Prus et al. [195]
Cocaine	3.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Terry et al. [173] Witkin et al. [179]
Cocaine	2.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Kantak et al. [196]
Cocaine	3.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Kantak et al. [197]
Cocaine	2.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Schechter [133]
Cocaine	3.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	No	Costanza et al. [198]
Diazepam	1.0 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Tang & Franklin [199]
Ethanol	750 mg/kg *vs.* vehicle 1500 mg/kg *vs.* vehicle	No	De Vry & Slangen [194]
Ethanol	1.0 g/kg *vs.* vehicle 2.0 g/kg *vs.* vehicle	Yes	Colombo et al. [200]
Ethanol	1.0 g/kg *vs.* vehicle 1.5 g/kg *vs.* vehicle 2.0 g/kg *vs.* vehicle	Yes	Grant et al. [201]
Ethanol	1.0 g/kg *vs.* vehicle 1.5 g/kg *vs.* vehicle 2.0 g/kg *vs.* vehicle	Yes	Gatto & Grant [202]
Ethanol	1.0 g/kg *vs.* vehicle 2.0 g/kg *vs.* vehicle	Yes	Quertemont et al. [203]

TABLE 3-6. (*Continued*)

Training Drug	Training Dose Groups	Difference(s)	Reference
(+)LSD[b]	0.02 mg/kg *vs.* vehicle 0.08 mg/kg *vs.* vehicle 0.32 mg/kg *vs.* vehicle	Yes	White & Appel [175]
Morphine	0.3 mg/kg *vs.* vehicle 10 mg/kg *vs.* vehicle	Yes	Gianutsos & Lal [204]
Morphine	1.75 mg/kg *vs.* vehicle 5.6 mg/kg *vs.* vehicle	Yes	Shannon & Holtzman [205]
Morphine	3.2 mg/kg *vs.* vehicle 5.6 mg/kg *vs.* vehicle	Yes	Young et al. [206]
(−)Nicotine	0.1 mg/kg *vs.* vehicle 0.2 mg/kg *vs.* vehicle 0.4 mg/kg *vs.* vehicle	Yes	Stolerman et al. [207]
5-OMe DMT[c]	1.5 mg/kg *vs.* vehicle 3.0 mg/kg *vs.* vehicle	Yes	Young et al. [208]
Pentobarbital	5.0 mg/kg *vs.* vehicle 15 mg/kg *vs.* vehicle	No	De Vry & Slangen [194]
Δ^9-THC[d]	1.8 mg/kg *vs.* vehicle 3.0 mg/kg *vs.* vehicle 5.6 mg/kg *vs.* vehicle	Yes	Järbe et al. [209]

[a]Ethyl-β-carboline-3-carboxylate.
[b](+)Lysergic Acid Diethylamide.
[c]5-Methoxy-*N*,*N*-dimethyltryptamine.
[d]Δ^9-Tetrahydrocannabinol.

to *S*(+)amphetamine and apomorphine [204]. Further, the stimulus effects of 1.75 mg/kg or 3.2 mg/kg of morphine *versus* 5.6 mg/kg of morphine in rats were shown to determine the likelihood that other agents would generalize, not generalize, or antagonize the stimulus effects of morphine [e.g., 205, 206]. In other studies, Young et al. [208] presented data that the stimulus effects of 1.5 mg/kg, but not 3.0 mg/kg, of 5-methoxy-*N*,*N*-dimethyltryptamine (5-OMe DMT, a hallucinogenic tryptamine) were blocked by the nonselective 5-HT receptor antagonist BC-105 (pizotyline). Mumford and Holtzman [192] demonstrated that the discriminative stimulus effects of a relatively low dose of 10 mg/kg of caffeine were mediated primarily by catecholamine mechanisms, whereas those of 56 mg/kg of caffeine were mediated by an unknown mechanism that was unlikely to include effects of the catecholamine system. Further, the discriminative stimulus effects of a relatively low dose of 2.0 mg/kg or 3.0 mg/kg of cocaine have been shown to be markedly different from those of 10 mg/kg of cocaine [173, 179, 196; but see 198]. Indeed, those same doses of cocaine were shown to be a critical factor in the evaluation of interactions of cocaine and various opioid agents [197]. Taken together, the results of such studies corroborate the argument that the dose of training drug can determine the "distinctiveness" of discriminative stimuli.

f. "Steep" Dose Response Functions *An important issue of data analysis concerns dose response functions that display a steep slope, which indicates that an agent exerts its effects over a narrow "window" of doses*. Historically, bioassays of in vivo activity (drug discrimination studies included) have tested agents over a relatively wide range of doses. In many cases, investigators have chosen to test a $\log_{10}$ series of doses for a drug such as 0.3, 1.0, 3.0, 10, and 30 mg/kg, as originally suggested by Turner [210]. If an investigator was assured that the stimulus effects of a training drug or test agent were qualitatively/mechanistically similar across all doses, then this would be an appropriate approach for the selection of doses. In some instances, however, a more complex relationship between dose and percent drug-appropriate responding is seen, such as an inverted U-shaped function. As noted previously, few drugs exert only one pharmacological (including stimulus) effect, which may help to explain (at least in part) nonlinear dose response functions. For example, in some cases, doses of a drug may produce proportionate increases in percent drug-appropriate responding up to a certain level but, then, "too large a leap" in an administered dose produces a decrease in the percent of responding on the drug-designated lever (or a behaviorally disruptive effect with no responding at all). Such results may indicate that the higher dose produces, to some degree, qualitatively different stimulus effects that are unlike those of the dose of training drug and, consequently, subjects respond less on the drug-designated lever (see Section F: Data Analysis and Interpretations). The key question arises, however, of where the demarcation lies between doses that exert qualitatively or mechanistically different discriminative stimuli. In a number of studies listed in Table 3-6 the difference between doses is not large and a seemingly more prudent approach to the selection of doses, in comparison to the full increments in log dose suggested by Turner [210], would be to include ⅛, ¼, ½, or ¾ increments in doses between log units. This type of situation has been observed with agents where the difference in doses has been quite small between a vehicle-like response, some degree of drug-like responding, and/or a disruptive effect. For example, Figure 3-12 displays data obtained from rats trained to discriminate the stimulus effects of 1.0 mg/kg of DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane) *versus* vehicle [211]. Tests of DOM-stimulus generalization were conducted *initially* to 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg of *R*(−)MDA (*R*(−)-1-(3,4-methylenedioxyphenyl)-2-aminopropane); percent DOM-appropriate responding of 25%, 60%, and behavioral disruption of responding were noted, respectively. If the study had been terminated at this point, then it might have been concluded that DOM stimulus generalization did not occur completely to *R*(−)MDA. However, the acknowledgment that significant drug-appropriate responding can occur within a very narrow range of doses led to *follow-up* investigations of 1.5 mg/kg and 1.75 mg/kg of *R*(−)MDA; percent DOM-appropriate responding of 75% and 94% were noted, respectively (Figure 3-12). In another example, Colombo et al. [200] trained rats to discriminate either 1 g/kg of ethanol or 300 mg/kg of GHB (a.k.a. *gamma*-hydroxybutyric acid, 4-hydroxybutanoic acid, or sodium oxybate) from vehicle and demonstrated that cross-generalization occurred between the two training stimuli only within a narrow range of doses of each agent. Such results indicated that the slope of the dose response curve is a variable that must be considered in tests of stimulus generalization (and tests of antagonism). A steep dose response curve, in comparison to one that is shallow

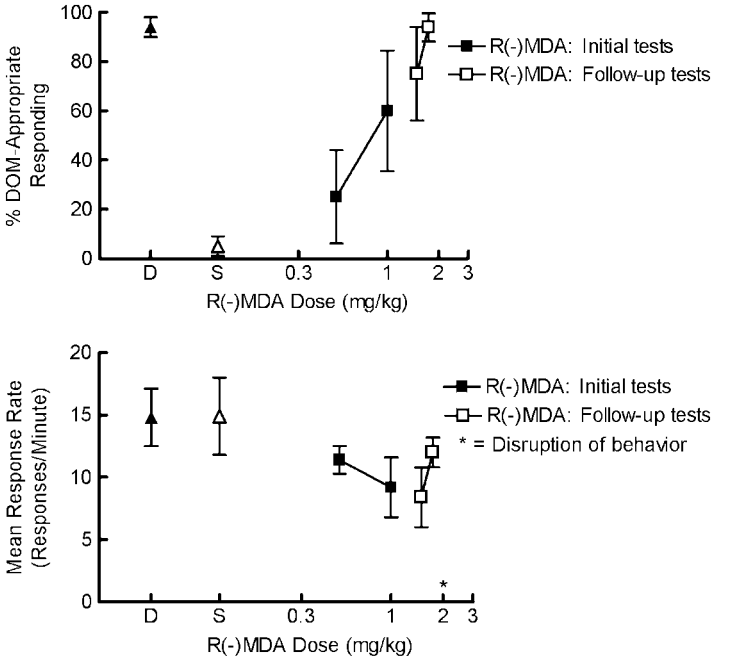

Figure 3-12. Effects of R(–)MDA in rats trained to discriminate the stimulus effects of 1.0 mg/kg of DOM versus vehicle. Ordinate: Data points represent group means (± S.E.M.) of % DOM-appropriate lever responding (top figure) and response rate data (bottom figure) after the administration of various doses of R(–)MDA. Tests were conducted initially to 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg of R(–)MDA; percent DOM-appropriate responding of 25%, 60%, and behavioral disruption (*) of responding (i.e., little or no responding) were noted (Figure 3-12: both graphs), respectively. Follow-up tests with 1.5 mg/kg and 1.75 mg/kg of R(–)MDA resulted in percent DOM-appropriate responding of 75% and 94% respectively. D and S represent percent DOM-appropriate responding (top figure) or responses/minute (bottom figure) following the training dose of DOM or saline, respectively.

(e.g., see Figure 3-9, bottom figure: pentobarbital stimulus generalization to oxazepam), indicates that there is a smaller difference between the dose that produces a minimal or moderate degree of percent drug-appropriate responding and the dose that results in complete stimulus generalization. The steeper the slope, the smaller the increase in dose required to go from minimal to maximal percent drug-appropriate responding.

g. Pre-session Injection Interval (PSII) *An issue of methodology that requires consideration is that different pre-session injection intervals (PSIIs) might exert a significant influence on the results obtained in tests of stimulus generalization.*

For example, Glennon et al. [212] trained rats to discriminate the stimulus effects of 1.0 mg/kg of *S*(+)amphetamine from vehicle (15 minute PSII). In tests of stimulus generalization, PMA (1-(4-methoxyphenyl)-2-aminopropane) was examined initially at the standard PSII and then at an extended PSII of 60 minutes; *S*(+)amphetamine-appropriate responding reached a maximum of 62% at 15 minute PSII (with an inverted U-shaped dose response) and a maximum of 20% at 60 minute PSII. However, the use of a 5 minute PSII resulted in complete *S*(+)amphetamine-appropriate responding to PMA (Figure 3-13).

Thus, PMA appears to produce an "almost immediate" *S*(+)amphetamine-like stimulus effect, which is not inconsistent with a reported "transient" motor stimulant effect of PMA in rats [e.g., 213]. In another example, the stimulus effects of the trans (4*R*, 5*R*) isomer of 4-methylaminorex ("U4EUh"), a designer drug, have been shown to be influenced significantly by PSII and those data are described in Chapter 4, Figures 4-27 and 4-28. Such results indicate that PSII, just as dose of training drug, can be a critical variable in the determination of a stimulus effect; when PSII is varied, different degrees of stimulus generalization might emerge.

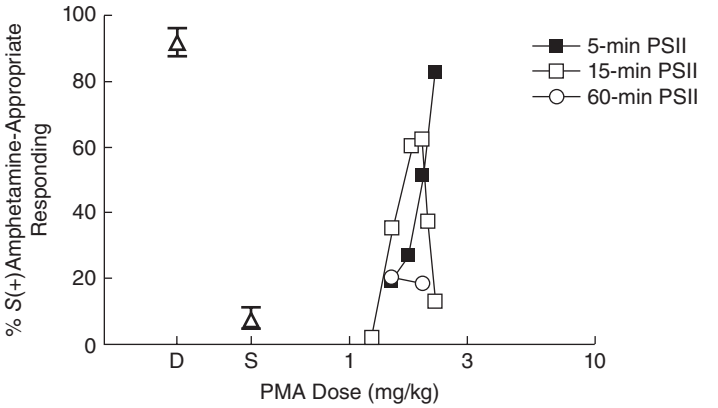

Figure 3-13. Effects of PMA, examined at various PSIIs, in rats to discriminate the stimulus effects of 1.0 mg/kg of S(+)amphetamine versus vehicle (15 minute PSII). Ordinate: Data points represent group means (S.E.M. not shown for purpose of clarity) of percent S(+) amphetamine-appropriate lever responding after the administration of various doses of PMA. PMA was examined initially at the standard PSII (i.e., 15 minutes) and then at an extended PSII of 60 minutes; S(+)amphetamine-appropriate responding reached a maximum of 62% at 15 minutes PSII (with an inverted-U-shaped dose response) and a maximum of 20% at 60 minutes PSII. However, a 5 minute PSII resulted in complete S(+)amphetamine-appropriate responding to PMA. D and S represent percent S(+)amphetamine-appropriate responding following the training doses of 1.0 mg/kg of S(+)amphetamine and saline vehicle, respectively.

h. Metabolism In a typical drug discrimination experiment, a subject is administered an agent parenterally and it is absorbed, distributed, and excreted. Most drugs, however, are not excreted unchanged by the organism but undergo metabolism or biotransformation. In many cases, this metabolism results in the creation of a chemical entity that is more water soluble than the "active" agent and therefore is more easily excreted by the kidneys. Thus, a drug that is quite *lipophilic* (can penetrate cell membranes to reach sites of action) may be metabolized to an agent that is more *hydrophilic*, which will increase the likelihood of its excretion. Typically, drugs are excreted as metabolites and/or in unchanged forms. Importantly, metabolism of a drug may result in an active drug that is converted to an inactive, somewhat less potent, equipotent, or even more potent (active) agent.

Drug metabolism reactions are carried out by enzyme systems that, for the most part, can be grouped into two general categories: phase I oxidative or reductive enzymes and phase II conjugative enzymes. Phase I reactions usually, but not always, precede phase II reactions. The most common phase I reactions are oxidative processes that involve cytochrome P450 (CYP 450) enzymes, a super-family of proteins found in all living organisms. Such reactions can occur by 1) CYP 450-dependent oxidations such as *aliphatic- and aromatic-hydroxylation* (e.g., barbiturates and propranolol, respectively), *N-dealkylation* (e.g., methamphetamine), *O-dealkylation* (e.g., codeine), *S-oxidation* (e.g., phenothiazine analogs), or *N-oxidation* (e.g., morphine, imipramine); 2) CYP 450-independent oxidations such as *alcohol- or aldehyde-dehydrogenation* (e.g., ethanol), *decarboxylation* (e.g., levodopa), or *oxidative deamination* of, for example, monoamines such as norepinephrine, epinephrine, and serotonin, which are catalyzed by monoamine oxidase type A (MAO-A), phenethylamine that is catalyzed by monoamine oxidase type B (MAO-B), and dopamine, which is inactivated by both types of MAO; 3) *ester- or amide-hydrolysis*, which usually involves enzymes such as esterases, amidases, and proteases that generate hydroxyl or amine groups that are suitable for phase II conjugation. Typically, esters undergo hydrolysis more quickly than their corresponding amides and, as such, amides tend to be longer acting than esters (e.g., procainamide *versus* procaine, respectively); or 4) *reductive reactions* such as nitro- or carbonyl-reductions and dehalogenation (e.g., halothane). Phase II reactions, usually known as conjugation reactions [e.g., with glucuronic acid, sulfonates (usually referred to as sulfation), glutathione, or amino acids], typically involve the attachment of a more polar molecule (i.e., hydrophilic group) to the original drug molecule to increase water solubility, thereby permitting more rapid drug excretion. Substrates for these reactions include both metabolites of phase I reactions and agents that already contain substituent groups appropriate for conjugation reactions such as carboxyl (-COOH), hydroxyl (-OH), amino (NH_2), and sulfhydryl (-SH) moieties.

Drug discrimination studies have evaluated metabolites of many different agents and these can be searched through the Drug Discrimination bibliography (http://www.drugref.org); some examples are discussed in various contexts in Chapters 3 (e.g., *S*(+)amphetamine and diazepam), 4 (e.g., *S*(+)amphetamine, DOM, and diazepam), and 5 (e.g., benzodiazepines).

F. DATA ANALYSIS AND INTERPRETATIONS

1. Complete Generalization

In general, the occurrence of *stimulus generalization* indicates that percent drug-appropriate responding is likely to be emitted in the presence of the stimulus effects of a test agent that are, to some degree, similar to the effects of the dose of training drug to which drug-appropriate responding was reinforced previously; responding will be emitted on the vehicle-designated lever under stimulus conditions that differ from those of the dose of training drug. Thus, stimulus generalization is a relative phenomenon such that the more the pharmacological features of a dose of test agent resemble the stimulus conditions present during training, the greater the probability that the trained response (i.e., perhaps ≥80% drug-appropriate responding) will be emitted. Figure 3-9 displayed examples of stimulus generalization in subjects trained to discriminate either diazepam (3.0 mg/kg) or pentobarbital (5.0 mg/kg) from vehicle. Stimulus generalization occurred when the animals performed ≥80% of their responses on the drug-appropriate lever after being administered doses of benzodiazepine or barbiturate analogs as test drugs. Furthermore, ED_{50} values were calculated (Table 3-5), which reflect the dose at which the animals would be expected to make 50% of their responses on the drug-appropriate lever [125, 126]. *An additional and important point, however, is that stimulus generalization between a training drug and a test compound is simply evidence that both agents can produce a similar stimulus effect but it is not necessarily accurate to conclude that they do so through an identical mechanism of action* (see Stimulus Antagonism below and Chapter 6).

2. Vehicle-Like Responding and Partial Generalization

In addition to complete stimulus generalization, an investigator will encounter two other types of test results: *partial generalization* and responding on the vehicle-designated lever (i.e., no generalization). A key factor in the interpretation of these types of results can be found in (often forgotten and/or ignored) early studies of drug discrimination that established the specificity of numerous drugs as discriminative stimuli (e.g., see Table 3-7). In those studies, when subjects (trained to a dose of drug *versus* vehicle) were administered behaviorally active doses of drugs from other drug classes, *they responded predominantly—if not exclusively—on the vehicle-designated lever*. In other words, when subjects are administered doses of a test agent that produce effects that are known to be dissimilar to both the dose of training drug and vehicle treatment, they do not ("guess" and) divide their responses equally (i.e., 50% drug-appropriate responding) between the two levers. Nevertheless, some studies (regardless of the established database) cling to the idea that when a dose of a test drug produces 50% drug-appropriate responding, the conclusion is that the dose of test drug produces stimulus effects that are not like those of the dose of training drug and not like the vehicle state.[*]

[*]Such a conclusion leads to additional confusion because it indicates that the definition of an ED_{50} value is the calculated dose at which subjects' responding is *not drug-like* or *vehicle-like*.

TABLE 3-7. Examples of some drug discrimination studies that established the specificity of pharmacological agents as stimuli

Training Drug	Test Agent	Dose Range Tested	Highest % of Drug-Appropriate Responding	Reference
Arecoline (1.5 mg/kg)	Morphine	2.0–8.0 mg/kg	13%	Jung et al. [217]
Bupropion (40 mg/kg)	(+)LSD[a]	0.08–0.32 mg/kg	20%	Blitzer & Becker [218]
	Clonidine	0.08–0.32 mg/kg	20%	
DOM[b] (1.0 mg/kg)	*S*(+)amphetamine	1.0–3.0 mg/kg	26%	Glennon et al. [211]
Fenfluramine (1.0 mg/kg)	*S*(+)amphetamine	0.25–2.0 mg/kg	17%	White & Appel [219]
GHB[c] (200 mg/kg)	*S*(+)amphetamine	0.8–3.0 mg/kg	25%	Winter [39]
Mescaline (10 mg/kg)	*S*(+)amphetamine	0.1–1.0 mg/kg	10%	Winter [220]
	Cocaine	3.0–30 mg/kg	15%	
Midazolam (0.4 mg/kg)	(−)Nicotine	0.05–0.4 mg/kg	<10%	Garcha et al. [55]
	S(+)amphetamine	0.1–0.8 mg/kg	<10%	
	Morphine	0.5–4.0 mg/kg	<10%	
Morphine (10 mg/kg)	*S*(+)amphetamine	0.8–3.2 mg/kg	0%	Gianutsos & Lal [204]
Morphine (3.0 mg/kg)	Pentobarbital	1.0–17.5 mg/kg	15%	Shannon & Holtzman [221]
Morphine (3.0 mg/kg)	Ketamine	1.0–30 mg/kg	20%	Shannon & Holtzman [160]
	Mescaline	3.0–100 mg/kg	0%	
	Physostigmine	0.03–1.0 mg/kg	15%	
Pentobarbital (5 or 10 mg/kg)	Morphine	1.0–10 mg/kg	2%	Herling et al. [69]
Phencyclidine (3.2 mg/kg)	Methylphenidate	3.2–32 mg/kg	12%	Browne [222]
	Cocaine	3.2–10 mg/kg	0%	
	S(+)amphetamine	1.0–1.78 mg/kg	0%	
Physostigmine (0.10 mg/kg)	(−)Nicotine	0.10–0.80 mg/kg	0%	Jung et al. [223]

In these studies, when subjects (trained to a dose of training drug versus vehicle) were administered behaviorally active doses of test agents from other drug classes, they responded predominantly—if not exclusively—on the vehicle-designated lever (i.e., percent drug-appropriate lever responding was low).
[a](+)Lysergic acid diethylamide.
[b]1-(2,5-Dimethoxy-4-methylphenyl)-2-aminopropane.
[c]*gamma*-Hydroxybutyric acid, 4-hydroxybutanoic acid, sodium oxybate.

However, the literature indicates quite clearly that, in such cases, subjects do not respond along a general continuum of "any drug effect" versus "not-drugged" state, such that "random (i.e., 50%)" drug-appropriate responding occurs (Table 3-7). Instead, subjects concentrate their responses on the vehicle-designated lever. For example, Shannon and Holtzman [160] trained animals to discriminate 3.0 mg/kg of morphine from saline and found that test doses between 3.0 mg/kg and 100 mg/kg of mescaline,

a hallucinogen, produced (a maximum of) 0% morphine-appropriate responding; such doses (especially up to 100 mg/kg) of mescaline are clearly not inert. That type of result (and other results noted in Table 3-7) has been termed "transfer (or stimulus generalization) test over-inclusiveness" by Overton [214] or "third-state hypothesis" by Frey and Winter [215]: that is, subjects trained to discriminate a dose of training drug will respond in a manner that is appropriate for the vehicle condition (i.e., press the vehicle-designated lever) under drug effects that are unlike those of the training drug or vehicle. Could that type of vehicle-like responding be an instance of "latent learning"? That is, during training, have subjects somehow established (i.e., "learned") the vehicle-designated lever as a default choice and respond on that lever in stimulus generalization tests when the effects of the test agent are not like those of the dose of the training drug or vehicle? If so, such "learning" is an uncharted area of investigation for practitioners of drug discrimination because it was not dependent upon the presentation of positive or negative reinforcement (i.e., in relation to obtaining "reward" or avoidance/escape from shock) during training. *Nevertheless, and simply stated, if the effects of test agents are not like those of the stimulus effects of the dose of training drug nor like the vehicle (i.e., inert) condition, then the vehicle-designated lever appears to serve as a* default *lever choice. Thus, when doses of a test drug produce vehicle-like responding (i.e., ≤20% drug-appropriate responding), the results may, but do not necessarily, indicate that the dose of test drug is inert. Such results only indicate that the effects of the test drug are "different" from those produced by the dose of training drug.*

The latter concept is illustrated graphically in Figure 3-14, with rats trained to discriminate 3.0 mg/kg of diazepam, a benzodiazepine agent that is prescribed for the treatment of anxiety, from vehicle.

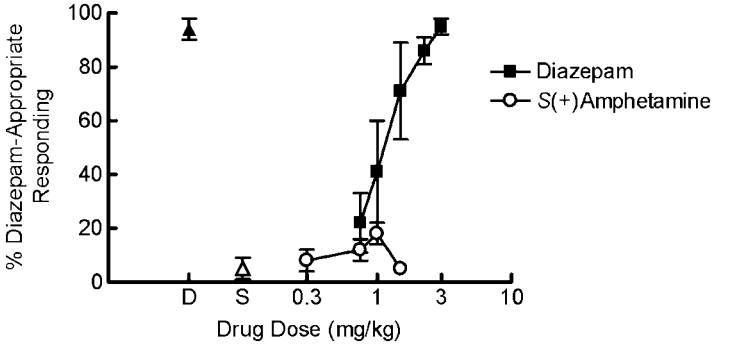

Figure 3-14. Effects of S(+)amphetamine in rats to discriminate the stimulus effects of 3.0 mg/kg of diazepam versus vehicle. Ordinate: Data points represent group means (± S.E.M.) of percent diazepam-appropriate lever responding after the administration of various doses of S(+)amphetamine. The administration of 0.30 mg/kg to 1.5 mg/kg of S(+)-amphetamine produced vehicle-like responding (i.e., maximal 18% diazepam-appropriate lever responding at 1.0 mg/kg), while the administration of 2.0 mg/kg to 3.0 mg/kg of S(+)-amphetamine produced disruption of behavior (i.e., no responding; data not depicted). D and S represent percent diazepam-appropriate responding following the training dose of 3.0 mg/kg of diazepam and saline vehicle, respectively.

First, lower doses of diazepam were administered to produce a dose response function; subjects responded progressively less on the diazepam-appropriate lever. In contrast, the administration of 0.3 mg/kg to 1.5 mg/kg of *S*(+)-amphetamine, a CNS stimulant, to the diazepam-trained animals produced vehicle-like responding (i.e., maximal 18% diazepam-appropriate lever responding at 1.0 mg/kg), while the administration of 2.0 mg/kg to 3.0 mg/kg of *S*(+)-amphetamine produced disruption of behavior (i.e., no responding; data not depicted). Clearly, the low percentage of diazepam-appropriate lever responding that followed *S*(+)amphetamine administration indicates that the stimulus effects produced by 3.0 mg/kg of diazepam are quite different from those produced by *S*(+)-amphetamine. Moreover, those (tested) doses of *S*(+)-amphetamine can serve as discriminative stimuli, which indicates that they are not inert [e.g., see 216].

When subjects are administered a dose of test drug and do perform 50% drug-appropriate responding, such results indicate *partial* or *intermediate stimulus generalization* in that drug-appropriate responding is somewhat like the dose of training drug and somewhat "vehicle-like"; recall, the vehicle-designated lever is the default lever and subjects press it under the vehicle (i.e., inert) condition or when the effect of the test agent is dissimilar from that of the dose of training drug. Thus, if some degree of similarity exists between the stimulus effects of the training drug and those of the test drug, then percent drug-appropriate responding may occur above the level of vehicle (≥20%). Subjects likely respond according to conditions of "some degree of similarity of stimulus effects of dose of training drug to those of test agent" (respond to some degree on drug-designated lever) *versus* "no-drug effect or different drug effect" (respond to some degree on vehicle-designated lever). Under such a view, subjects might learn stimulus effects as a consequence of the interactions of the effects of the dose of training drug at neurotransmitter receptor systems, behavior, and reinforcement. Further, stimulus effects might occur as a dimension of difference that allows pharmacological effects of the agent to be distinguished from one another. Consequently, the "distinctive" pharmacological features of the dose of the training drug are "promoted" (especially in interactions with presentations of reinforcement) within the subject when procedures are implemented to establish the drug as a discriminative stimulus. As subjects become more exposed to those aspects of the actions of the dose of drug that serve as the stimulus during training, the more likely they are to learn those key pharmacological features, and the more accurate becomes their percent drug-appropriate responding. For instance, a psychoactive drug may interact with more than one monoamine neurotransmitter system, but one of those systems may predominate in terms of subjects' ability to discriminate that chemical entity (or generalize to a related analog). In other words, subjects might employ (primarily) one of the amine systems as a means to detect the presence of stimulus effects even though that drug may affect other systems as well (see Chapter 6). An *S*(+)amphetamine (e.g., typically 1.0 mg/kg) stimulus, for example, seems to be mediated primarily through central catecholamine (mainly dopamine) pathways but it also can affect or be affected by serotonin systems, as evidenced by a leftward shift of the dose response of *S*(+)amphetamine after administration of doses of *S*(+)amphetamine in combination with fixed doses of the serotonin 5-$HT_{1A/7}$ receptor agent 8-OH DPAT; that is, 8-OH DPAT made *S*(+)amphetamine more *S*(+)

amphetamine-like to the *S*(+)amphetamine-trained subjects [e.g., 216, 224; see Chapter 6]. *This perspective of drugs as discriminative stimuli represents a significant advance in the field as compared, for example, to an early commentary of "the drug is the stimulus," when researchers in the field were unable to specify with any, or only a slight, degree of confidence the receptor interactions involved in the mediation of drug stimuli* [e.g., see 225; commentary by Catania]. *Moreover, this view is consistent with current neuroscience that incorporates the discoveries of neurotransmitter receptor families, family receptor subtypes, and inventions of relatively site-selective agents that have occurred over the last 35 years.*

In other results of tests, some degree of stimulus generalization (substitution) may occur as long as the pharmacological features that are present in the test stimulus "match" those that have been learned during training; that is, the test stimulus may be recognized partially or almost completely. Consequently, partial generalization has been stated (reasonably and consistently) to have occurred when subjects, after being administered a thorough dose-response test, perform approximately (technically 21% to 79% but usually) 40% to 65% of their responses on the drug-appropriate lever [e.g., 216, 226–228]. In Figure 3-15 the stimulus effects of diazepam (3.0 mg/kg), which are likely mediated via an allosteric modification of the $GABA_A$ receptor subtype, generalized partially to buspirone, a serotonin 5-HT_{1A} receptor (partial) agonist anxiolytic agent that is structurally unrelated to diazepam; maximal 43% diazepam-appropriate lever responding occurred at 3.0 mg/kg and disruption of behavior was observed at doses between 3.25 mg/kg to 10 mg/kg of buspirone (disruption data not shown).

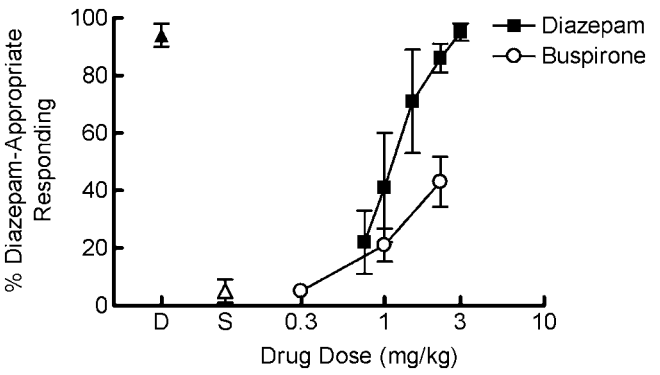

Figure 3-15. Effects of buspirone in rats to discriminate the stimulus effects of 3.0 mg/kg of diazepam versus vehicle. Ordinate: Data points represent group means (± S.E.M.) of percent diazepam-appropriate lever responding after the administration of various doses of buspirone. The administration of 3.0 mg/kg of buspirone produced maximal 43% diazepam-appropriate lever responding while the administration of doses between 3.25 mg/kg to 10 mg/kg of buspirone produced disruption of behavior (i.e., no responding; data not depicted). D and S represent percent diazepam-appropriate responding following the training dose of 3.0 mg/kg of diazepam and saline vehicle, respectively.

The lack of complete substitution between the stimulus effects of diazepam and buspirone was not unexpected, however, because complete stimulus generalization also did not occur between buspirone and oxazepam when either drug was used as the training stimulus [9]. Thus, a diazepam stimulus may generalize partially to buspirone because there may be some degree of stimulus effects that are common to both diazepam and buspirone. However, the overlap of their stimulus effects is insufficient to result in complete stimulus generalization.

The latter findings indicate that, even within a pharmacological class, drug discrimination procedures may differentiate among drugs that exert activity at different neurotransmitter receptors (also see Figure 3-21 below). Thus, both diazepam and buspirone have been shown to be active in conditioned and unconditioned behavioral procedures that are thought to be indicative of antianxiety-like action, such as conflict and marble-burying behaviors, respectively. In a widely used operant (conflict) procedure, a subject performs a *conditioned* response that results in both the presentation of reinforcement and punishment [e.g., 229–231]. Figure 3-16 presents the effects of

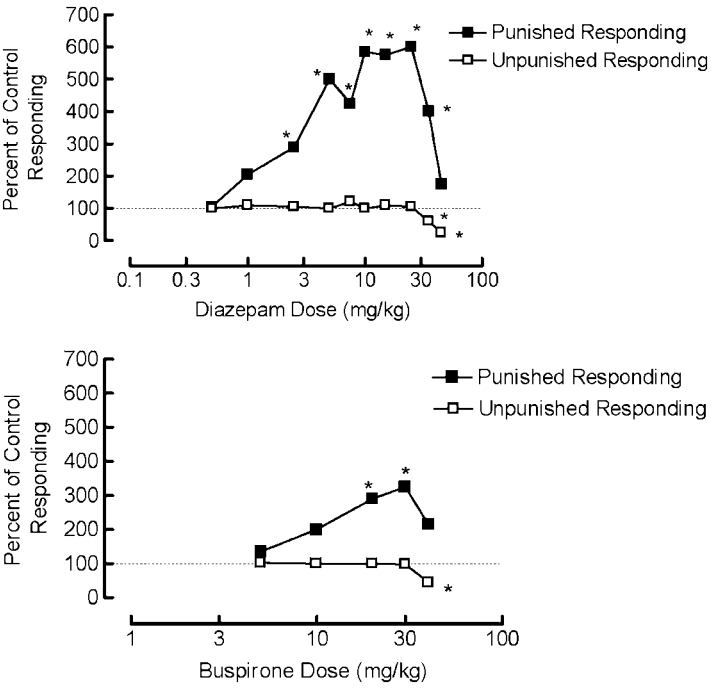

Figure 3-16. Dose effect function for diazepam (top graph) and buspirone (bottom graph) on punished fixed ratio 1 (solid squares) and unpunished variable interval 90 second (open squares) behavior in rats. Data points represent group means. The effect on treatment days is expressed as a percentage of the responses for the vehicle control day that preceded each treatment. The dashed horizontal line at 100% represents the control level. Symbols that are marked with an asterisk represent statistically significant ($p < 0.05$) increases in punished responding or decreases in unpunished responding.

diazepam (top figure) and buspirone (bottom figure) in a conflict procedure that involves a multiple variable-interval fixed-ratio (multiple VI-FR) schedule of reinforcement [232]. In the FR component, a brief electric shock coincided with the presentation of reinforcement (i.e., punished responding).

The ability of a drug to increase (or "disinhibit") responding that is suppressed by electric shock is used as an indicator of a potential anxiolytic-like effect; responding under the VI portion of the schedule is used as a control response. Diazepam produced dose dependent increases in punished responding (i.e., an anxiolytic-like effect) over a 10-fold range of doses (2.5–25 mg/kg). In comparison, buspirone also enhanced response rates significantly under punishment conditions with maximal increases in punished responding occurring between 20 mg/kg and 30 mg/kg.

Diazepam and buspirone also have been evaluated in procedures that use *unconditioned* behavior, such as object burying by mice, as an indication of anxiolytic-like activity [233, 234]. In one version of the test, mice are placed in cages that contain glass marbles that are evenly distributed, along the walls, on top of a layer of bedding material. Under vehicle conditions, rodents bury a substantial number (i.e., 65–75%) of the marbles. An anxiolytic-like effect is assumed from drug-induced decreases in marbles buried (i.e., left uncovered). Figure 3-17 reveals that mice treated with diazepam (3.0 mg/kg, 10 mg/kg, or 30 mg/kg) or buspirone (10 mg/kg or 30 mg/kg) exhibited dose-related antianxiety-like activity as reflected by statistically significant increases in the percentage of marbles left uncovered in cages.

Taken together, the results of diazepam and buspirone in the conflict, marble-burying, and drug discrimination procedures indicate that even though agents might share an anxiolytic-like effect as demonstrated by their anti-conflict or anti-burying activity, they might not necessarily share similar stimulus effects, which suggests a role for diverse neurochemical mechanisms to achieve the therapeutic treatment of anxiety.

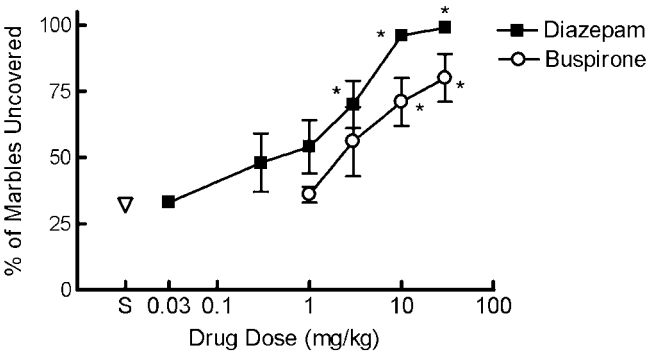

Figure 3-17. Dose effect functions for diazepam (solid squares) and buspirone (open circles) on marble burying behavior in mice. Data points represent group means (± S.E.M.) of percent of marbles that remained uncovered after doses of each agent or saline (S) vehicle. Symbols that are marked with an asterisk represent statistically significant ($p < 0.05$) increases from saline control.

An extrapolation may be that patients who suffer from anxiety might experience an antianxiety effect to each drug but perceive them, to some degree, quite differently. Indeed, that explanation is quite reasonable because humans have been successfully trained in a three-choice operant conditioning task to discriminate the stimulus effects of 15 mg/70 kg of buspirone *versus* 10 mg/70 kg of diazepam *versus* vehicle [see Table 3-2 and 99].

The aforementioned examples of vehicle-like responding and partial generalization suggest that subjects trained in a drug discrimination task (e.g., diazepam *versus* vehicle) respond on the drug-designated lever only when administered a test agent that produces some degree of stimulus effects (e.g., buspirone) that are similar to those of the dose of the training drug. If the test agent produces stimulus effects that are "inert' or *unlike* those of the training drug (e.g., *S*(+)amphetamine), then responding will occur on the vehicle-designated lever until doses of the test agent are administered that disrupt the subjects' lever response behavior (i.e., little or no responding).

One approach to the characterization or interpretation of partial generalization results involves the study of structure–activity relationships to determine whether closely related analogs have stimulus actions that are similar to those of already established agents. In other words, analogs are tested and compared in stimulus generalization tests to one or more training drugs. For example, the phenylisopropylamine (PIA) chemical structure is found in certain drugs of abuse and the appearance and location of certain substituent groups seem to account for differences in mechanism of action and, consequently, stimulus effects. Such PIAs can be classified as 1) central stimulants, such as amphetamine, which are thought to exert their action via catecholamine mechanisms (e.g., release of dopamine); 2) hallucinogens, such as DOM (1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane), which are believed to act primarily via a serotonin (5-HT) mechanism (probably a 5-HT_{2A} receptor agonist effect); and 3) designer or "other" drugs, such as PMMA (*N*-methyl-1-(4-methoxyphenyl)-2-aminopropane), MDMA (*N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane; a.k.a. "Ecstasy," "XTC," "X"), and MDA (1-(3,4-methylenedioxyphenyl)-2-aminopropane; a.k.a. "Love Drug"), whose mechanisms of action have not yet been elucidated fully but are thought to involve interactions with the monoamines serotonin, norepinephrine, and/or dopamine [e.g., 185, 235–237]. Moreover, a classification model has been established to account for the stimulus effects produced by these agents (e.g., 72, 238]. This scheme proposes that the three prototype agents (i.e., amphetamine, DOM, and PMMA) exert distinct, yet *overlapping*, types of stimulus effects and structure-activity relationships; Chapters 5 (Section A) and 6 (Sections A–D) provide detailed discussions of these representations and interrelationships. Of interest here, however, is that the model also may provide insights, but not yet completely definitive evidence, into the occurrence of partial generalization. For example, rats trained to discriminate either *S*(+)amphetamine (1.0 mg/kg), DOM (1.0 mg/kg), or PMMA (1.25 mg/kg) *versus* vehicle have been tested with various methoxy-substituted analogs of the basic phenylisopropylamine (i.e., amphetamine) structure, such as PMA (a monomethoxy derivative), 3,4 DMA (a dimethoxy analog), and 2,4,5 TMA (a trimethoxy derivative). In brief, PMA produced *S*(+)amphetamine-like stimulus effects and appears capable of producing some partial PMMA-like stimulus action (63% PMMA-appropriate responding) but does not exert

DOM-like stimulus effects (Figure 3-18, top graph). In comparison, 3,4 DMA produced partial DOM-like (46% DOM-appropriate responding) and partial PMMA-like (71% PMMA-appropriate responding) stimulus effects but does not produce *S*(+)amphetamine-like stimulus activity (Figure 3-18, middle graph). Lastly, 2,4,5 TMA produced DOM-like stimulus effects and partial *S*(+)amphetamine-like stimulus activity (46% *S*(+)

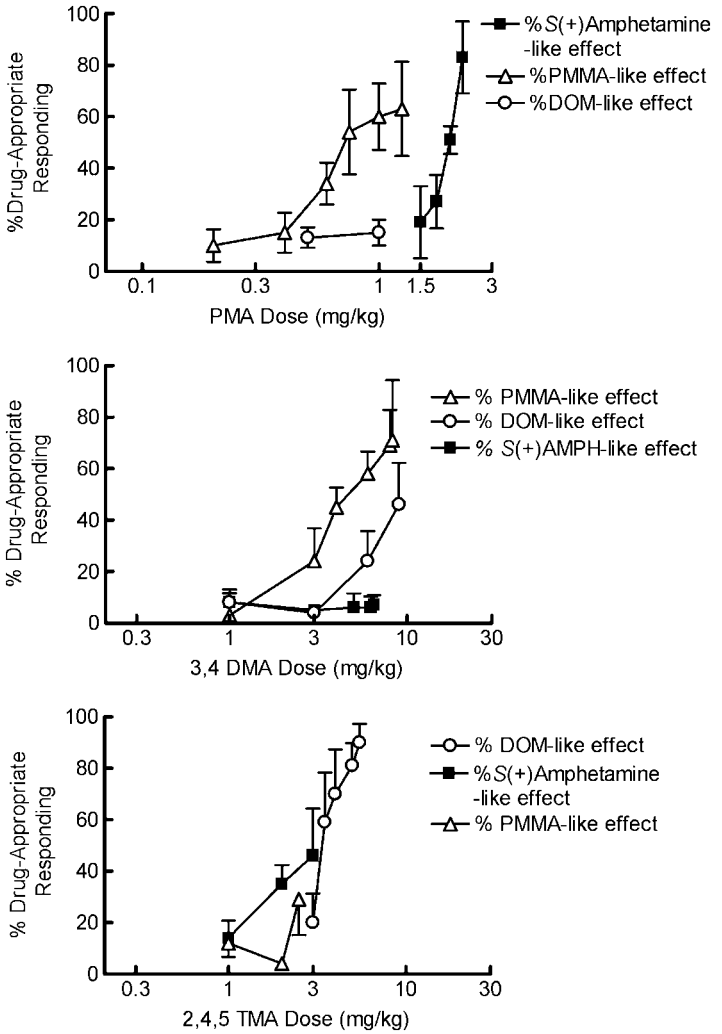

Figure 3-18. Dose-response effects of PMA (top), 3,4 DMA (middle), and 2,4,5 TMA (bottom) in rats trained to discriminate either 1.0 mg/kg of S(+)amphetamine, 1.25 mg/kg of PMMA, or 1.0 mg/kg of DOM versus vehicle. Ordinate: Data points represent group means (± S.E.M.) of % drug-appropriate lever responding after the administration of the test agents in each group of subjects. Abscissa: Dose of test agent plotted on a logarithmic scale.

amphetamine-appropriate responding) but does not exert PMMA-like stimulus effects (Figure 3-18, bottom graph).

Taken together, the results suggest that even though methoxy-substituted derivatives might exhibit a primary (substitution) stimulus effect, they also might exert (to various degrees) another type(s) of stimulus effect. Thus, PMA produced complete *S*(+) amphetamine-like stimulus effects and also produced some stimulus action that was PMMA-like. Similarly, 2,4,5 TMA produced complete DOM-like stimulus effects and also produced some stimulus activity that was *S*(+)amphetamine-like.

The latter findings of partial generalization are difficult to interpret precisely but might have occurred because there are stimulus effects that are common to both the dose of training drug and the test agent. However, complete stimulus generalization may not have occurred because the overlap of effects was incomplete. Indeed, the aforementioned classification model would support the idea that those partial generalizations were the result of stimulus effects that were *overlapping* to both the dose of training drug and the test agent. Thus, subjects in the PMMA- and *S*(+)amphetamine-trained groups might have indicated that low doses of PMA and 2,4,5 TMA, respectively, were similar to low doses of training drug. However, as the dose of test agent was increased, another (more prominent) kind of stimulus action emerged. As such, the model can explain partial generalization results as a similarity, to some degree, in stimulus properties of a dose of PIA training drug and doses of test agent. Importantly, the model does not preclude the possibility that those agents can exert an additional stimulus effect(s) or that a converse profile of stimulus generalization results would necessarily be observed; for example, 2,4,5 TMA-trained subjects would not necessarily have to exhibit complete stimulus generalization, partial generalization, and no generalization to DOM, *S*(+)amphetamine, and PMMA, respectively (also see discussion in Section E, # 2d above).

Lastly, a review of the drug discrimination literature indicates that partial generalization results have been (and are) an ongoing topic of debate/controversy in the field (e.g., see Chapter 16 by Colpaert). Such debate is healthy and is much preferred to outlooks that simply ignore, dismiss, or treat partial generalization results as some kind of obscure by-product of "inadequate methodology" in the assay.

3. Statistics

One of the most debated and controversial issues in drug discrimination experiments involves the use, partial use, misuse, or near lack of statistical analyses beyond those of mean percent drug-appropriate responding [(± standard deviation (S.D.) or ± standard error of the mean (S.E.M.)], ED_{50} or AD_{50} values (with 95% confidence limits), and mean response rate [e.g., responses per second or minute (± S.D. or S.E.M.)]. Statistical evaluations of drug discrimination data have included both parametric (e.g., ANOVA, Student's t-test) and nonparametric (e.g., Mann-Whitney, Kruskal-Wallis, and Friedman's) procedures [for review, see 239]. In drug discrimination experiments, the same subjects receive two or more different treatments and this design is termed within-subject, repeated-measures, or treatment-by-subjects and the parametric statistic of choice would be ANOVA followed by a post-hoc procedure (e.g.,

Student-Newman-Keuls, Dunnett's, or Bonferroni). It is important to note that drug discrimination procedures provide both quantitative and qualitative data about the relationship between the stimulus effects of a dose of training drug in relation to a dose of test agent. Some investigators argue that, based on the *quantitative* aspect of the assay, measurements demand the use of statistical tests. The argument is made that statistical tests will reduce "bias" in the analyses of data and provide a probability value that any observed differences in the study occurred by chance alone. Other investigators have argued that an (over) emphasis on *quantitative* analyses can lead to statistical inferences about the *qualitative* aspects, characteristics, or attributes between pharmacological agents that are ambiguous or equivocal (see Stimulus Generalization below).

Acquisition of the Discrimination: In a two-lever operant conditioning task, subjects are administered their dose of training drug or vehicle and during initial training sessions they typically divide their responses equally between the levers (e.g., see Figure 3-5). However, as training progresses with the treatments, subjects gradually learn to respond on the drug-designated lever when administered the dose of training drug and respond on the vehicle-designated lever when administered vehicle. Once the discrimination is learned, well-trained performers exhibit marked tendencies for (correct) responding on the appropriate lever; recall, assessment of subjects' performance in the discrimination task is defined by the *separation* between percentage drug-appropriate responding following the dose of training drug *versus* vehicle conditions (e.g., see Figure 3-5). During this process of learning, a parametric statistical test such as an analysis of variance (ANOVA) and/or a t-test can be performed at any given point in time to determine whether a statistically significant difference exists between the subjects' percent of drug-appropriate responding after their administration of dose of training drug versus vehicle. In Figure 3-5 (top graph), for example, subjects performance can be differentiated statistically (i.e., is significantly different) with a t-test for related measures at session 23; subjects response of 64% on the diazepam-appropriate lever following the administration of 3.0 mg/kg of diazepam is significantly different from their response of 30% on the diazepam-appropriate lever following the administration of vehicle ($t = 4.69$, $df = 11$, $p \leq 0.05$). However, experimenters typically train subjects far beyond and with a much stricter criterion than just the demonstration of a statistically significant difference in performance at some point during training. In most cases, subjects (individually and, thus, as a group) must attain and consistently maintain (e.g., 2–4 weeks) ≥80% of their total responses on the drug-appropriate lever after injection of their dose of training drug and ≤20% of their responses on the drug-appropriate lever after administration of vehicle. The argument is proffered that once subjects have attained and maintain such a stringent criterion, it serves little purpose to test for a statistically significant difference(s) between percent drug-appropriate responses after the dose of training drug *versus* percent drug-appropriate responses following vehicle treatment conditions. Moreover, if statistical t-tests are employed during training, caution is advised not to apply *multiple* two-sample comparisons between percent drug-appropriate responding following the dose of training drug *versus* vehicle without appropriate correction in probability (p) values because, as the number of comparisons increases, Type 1 error (falsely rejecting the null hypothesis) increases (i.e., repeated calculation of two-sample t-tests comparisons is not appropriate).

Tests of Stimulus Generalization (or Antagonism): Interestingly, when a substitution test results in complete stimulus generalization that is dose-related, the data typically contain an example of each type of stimulus generalization result: that is, a "low" dose of the test agent that produces percentage drug-appropriate responding that is close to *vehicle-like responding* (~20% drug-appropriate lever responding), an "intermediate" dose that produces ~40% to 65% drug-appropriate responding and indicates *partial generalization* (described above), and a "high" dose that produces ≥80% drug-appropriate responding and indicates a *complete stimulus generalization*. Some investigators have adopted those strict guidelines in the characterization of data obtained in generalization tests; furthermore, little argument has occurred over those definitions of complete stimulus generalization (or no antagonism) or vehicle-like responding (or antagonism) in that profile [e.g., 216, 226–228]. On the other hand, data that are characterized as partial stimulus generalization (or antagonism) have generated much controversy and led some investigators to analyses of discrimination data by parametric or nonparametric statistical procedures. Unfortunately, statistical inferences from those tests have produced controversial conclusions about the *qualitative* aspects, characteristics, or attributes between pharmacological agents.

For example, *partial stimulus generalization* has been defined statistically as "*the test agent produced percent drug-appropriate responding that was statistically (significantly) different from both the conditions of vehicle and the dose of training drug*." However, to state a statistical difference between training drug and test drug and then discuss similar (i.e., partial, but not fully complete) stimulus effects between them appears logically inconsistent.

Second, *complete stimulus generalization* has been defined statistically as "*the dose of training drug and the dose of test drug produced percent drug-appropriate responses that were not statistically different but those responses were statistically different from vehicle*." In such a scenario, if the dose of the test agent produced ≥80% drug-appropriate responding, then the test agent attained the criteria for the dose of training drug that was required for training and, thus, complete stimulus generalization is said to have occurred; statistical analysis seems to add little to the conclusion but some investigators prefer the "dual criteria" of ≥80% drug-appropriate responding and statistical significance. In another example, however, a test agent may produce a stimulus generalization result (e.g., 65% drug-appropriate responding) that is not statistically different from the result of the training drug (e.g., 80% drug-appropriate responding) but, even though both of those results may be statistically different from vehicle, investigators are not comfortable in terming the 65% drug-appropriate response "complete generalization." As such, investigators may be comfortable with the results of the quantitative (statistical) calculation(s) but be very uncomfortable about the qualitative conclusion. Investigators typically prefer to reserve the claim of stimulus generalization to a test agent that produces a group mean of ≥80% drug-appropriate responding; furthermore, and ideally, each member of the group will produce ≥80% drug-appropriate responding. Alternatively, the 65% drug-appropriate response could be called "partial generalization." However, to state no statistical difference (i.e., similarity) between the responses of the training drug and test drug and then discuss some degree of dissimilar stimulus effects between them, again, appears logically inconsistent.

A third major concern of statistical procedures is their failure to account for *behavioral disruption* (i.e., no responding) in the analyses. For example, an animal that fails to press a lever after being administered a dose of test agent in a stimulus generalization test cannot be assigned a percent score; recall, 0% drug-appropriate responding cannot be assigned because it has a different meaning (i.e., the animal pressed the saline-designated lever). Some investigators argue that statistical analysis (e.g., ANOVA) is robust enough to account for such missing data and that procedures are available by which "missing" data can be estimated from existing data (e.g., "weighted" or "unweighted" means) for subjects (indeed, some software programs automatically perform such operations for a within-subject design). However, *such extrapolations or interpolations have as a prerequisite that the loss of data is unrelated to the treatment conditions.* Clearly, subjects have not met that requirement when they fail to respond because of the effects of a dose of drug (i.e., treatment condition). In other words, a subject's drug-appropriate lever response is not available because the drug interfered with the ability of the performer to respond; statistical interpolation is (more) applicable, for example, to an animal that may have been (accidently) overfed or missed transport to the lab and was not tested. For further discussion of the requirements and methods for estimating data under such circumstances see Kirk [240], Myers [241], or Winer [242].

A more palpable description of such data that are "missing" (and unavailable) because the drug interfered with the performer's response in the discrimination task is that the effect should be acknowledged in the study and characterized qualitatively as "disruption." Statistically, however, one could (and some investigators do) ignore the disruptive effect of a dose(s) of drug, use only the data from very few animals (sometimes n = 1 or 2 out of 6 or 8 subjects that were tested) that respond (usually those few subjects have responded to a high degree on the drug-designated lever), and conclude (statistically) the occurrence of stimulus generalization. However, in such cases, it seems more appropriate and meaningful to characterize the effect as "disruption" rather than to promote a statistical calculation that, albeit can be performed, may be misleading.

Nonetheless, spirited debate continues about statistical analyses within the field of drug discrimination. However, the ultimate aim of the investigator who uses drug discrimination procedures is (or should be) the distribution of results in such terms that the people who want or need the information will understand it.

4. Time Course

Once established, the dose of a training agent may be characterized for time course of effect: latency of onset to action, time taken to reach maximum effect, and duration of stimulus action. The latter effects are determined mainly by rate of absorption, distribution, metabolism, and excretion of the drug. In drug discrimination studies, tests of time course are conducted by changes in the pre-session injection interval (i.e., PSII), alterations in the length of time between the administration of the dose of training drug and the beginning of a test session. The latency of onset to action refers to the time between the administration of the dose of training drug and the first indications of marked effects on subjects' drug-appropriate responding (e.g., >20% drug-appropriate responding).

The peak activity of a discriminative stimulus refers to the time interval that the drug exerts maximal percentage drug-appropriate responding (e.g., typically ~80% to 100% drug-appropriate responding). Lastly, the duration of action refers to the interval of time between onset to action and the occasion that the stimulus no longer exerts a notable percentage of drug-appropriate responding (e.g., return to ~20% drug-appropriate responding).

In an example discussed previously, rats were trained to discriminate the stimulus effects of 3.0 mg/kg of diazepam from vehicle, a dose-effect function was determined, and an ED_{50} value (1.2 mg/kg) was calculated (see Table 3-5). Figure 3-19 (top graph) shows the dose response of diazepam and stimulus generalization to several metabolites of diazepam. Specifically, the diazepam stimulus generalized in a dose related manner

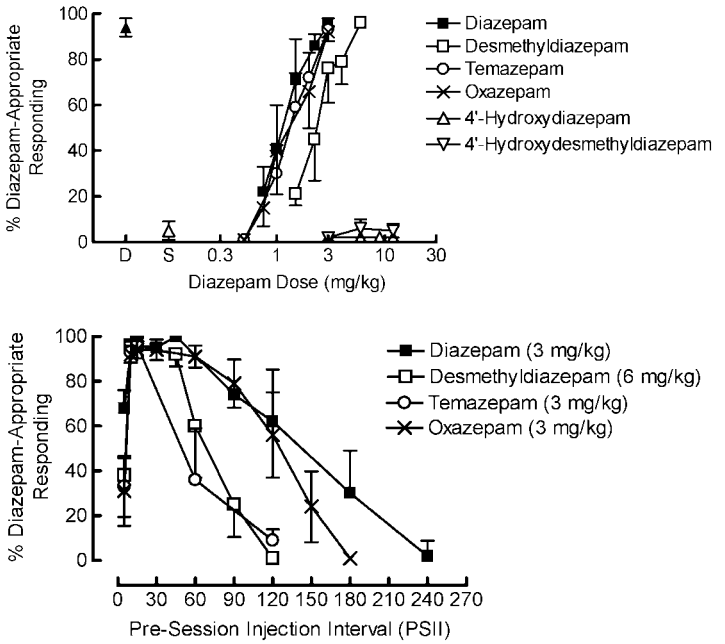

Figure 3-19. Results of stimulus generalization tests (top graph) with diazepam (closed squares), desmethyldiazepam (open squares), temazepam (open circle), oxazepam (x symbol), 4′-hydroxydiazepam (open triangle), and 4′-hydroxydesmethyldiazepam (open inverted triangle) in rats trained to discriminate 3.0 mg/kg of diazepam versus vehicle. Bottom graph depicts the results of time course studies (i.e., stimulus generalization tests with various PSIIs) with diazepam (3.0 mg/kg), desmethyldiazepam (6.0 mg/kg), temazepam (3.0 mg/kg), and oxazepam (3.0 mg/kg) in these same rats. Ordinate: Data points represent group means (± S.E.M.) of % diazepam-appropriate lever responding after the administration of doses of the test agents with the standard 15 minute PSII (top graph) or dose of each agent that produced stimulus generalization at various PSIIs (bottom graph). D and S (top figure) represent percent diazepam-appropriate responding following the training dose of 3.0 mg/kg of diazepam and saline vehicle, respectively.

to oxazepam (ED_{50} = 1.4 mg/kg), temazepam (ED_{50} = 1.4 mg/kg), and desmethyldiazepam (ED_{50} = 2.3 mg/kg) but not to 4'-hydroxydiazepam or 4'-hydroxydesmethyldiazepam [23]. Those results indicate that, in comparison to diazepam, the former three metabolites are relatively potent behaviorally and indicate the distinct possibility that they may contribute to the stimulus effect of diazepam.

Figure 3-19 (bottom graph) displays the time course effects of the stimulus effects of 3.0 mg/kg of diazepam, established with a PSII of 15 minutes, and evaluated with additional PSIIs of 5, 10, 30, 45, 90, 120, 180, and 240 minutes. The results indicated that the onset to effect of the diazepam stimulus was between 5 minutes and 15 minutes, peak activity occurred from 10 minutes to approximately 90 minutes, and duration of action transpired from 10 minutes to ~180 minutes. In addition, the diazepam-like time course effects were determined for the (active) metabolites of diazepam. These studies were conducted with the dose of each metabolite that produced complete stimulus generalization (i.e., ≥80% diazepam-appropriate responding) in the diazepam-trained animals: 6.0 mg/kg of desmethyldiazepam, 3.0 mg/kg of oxazepam, and 3.0 mg/kg of temazepam. Interestingly, in humans, diazepam is considered to have a relatively rapid onset of action and a relatively long half-life. In comparison, oxazepam and temazepam are considered to have relatively slower onsets of action and much shorter half-lives, relative to diazepam. Taken together, the time course studies of the stimulus effects of diazepam and the diazepam-like effects of the major metabolites of diazepam shown in Figure 3-19 (bottom) are not inconsistent with human data.

In another example, the time-course effects of the β-adrenergic agent *S*(–)propranolol, which is thought to sub-serve the effects of (±)propranolol, were examined in rats trained to discriminate the effects of 5.0 mg/kg of *S*(–)propranolol from vehicle [74]. In addition to the standard PSII of 15 minutes, the effects of PSIIs of 5, 60, 90, 120, and 150 minutes were studied (Figure 3-20).

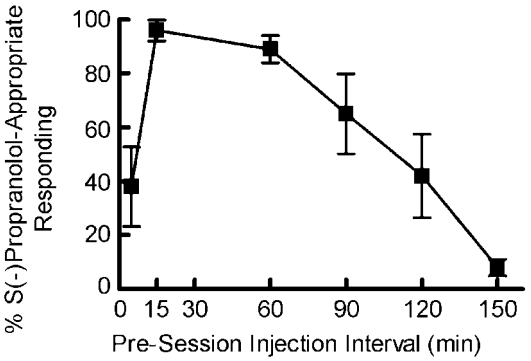

Figure 3-20. Time course of the S(–)propranolol (5.0 mg/kg) discriminative stimulus in rats trained to discriminate 5.0 mg/kg of S(–)propranolol versus saline vehicle. Ordinate: Data points represent group means (± S.E.M.) of % S(–)propranolol-appropriate lever responding after the administration of 5.0 mg/kg of S(–)propranolol administered at various PSIIs.

When the PSII was shortened to 5 minutes, the rats responded 40% on the *S*(−) propranolol designated lever. Following a PSII of 60 minutes, *S*(−)propranolol-appropriate responding (i.e., 91%) was similar to that observed after the standard PSII of 15 minutes. PSIIs subsequent to 60 minutes resulted in decreased percentages of total responses on the S(−)propranolol-appropriate lever; with PSIIs of 120 and 150 minutes, drug-designated lever responding was lowered to 39% and 11%, respectively. The latter results indicate that *S*(−)propranolol exerts a relatively fast onset of stimulus effects and a relatively short duration of effect. Moreover, those data parallel pharmacokinetic studies of propranolol in rats that indicate a rapid and near complete absorption after intraperitoneal administration and an observed peak plasma level that occurs within 1.0 hour but steadily declines thereafter [e.g., 243].

Clearly, familiarity with the time-course of action of the training drug or challenge agents in tests of stimulus generalization and/or stimulus antagonism (see below) is of great importance. Such studies can be employed to characterize the stimulus effects of a training drug, test agents, and can be used to ensure that further tests of the stimulus properties of a drug are not measured too long, or short, after drug administration. In fact, time course studies of training drugs and/or test agents are one of the most often performed procedures in drug discrimination experiments; 465 citations are noted in the Drug Discrimination bibliography (http://www.drugref.org) and PubMed (www.pubmed.gov).

5. Stimulus Antagonism

Research investigators have always seemed challenged by the determinants that allow drugs to serve as discriminative stimuli but are hidden from them in the dark folds of the brain. This does not stop pharmacological theorizing about the nature of these unseeable events. An effective plan to elucidate the mechanisms of action of psychoactive drugs is to study agents that block their effects. In drug discrimination studies, the rationale of such an approach is that the stimulus effects of the dose of the training agent will only be blocked by receptor antagonists that interfere with the mechanisms that form the basis of the discrimination. The results of antagonism tests, as with generalization tests, typically fall into one of three categories: 1) complete antagonism (i.e., vehicle-like responding); 2) partial antagonism (i.e., ~40–65% drug-appropriate responding); and 3) no antagonism (i.e., ≥80% drug-appropriate responding).

In cases of complete stimulus antagonism, subjects respond in a manner that is appropriate for the vehicle condition (i.e., press the vehicle-designated lever) after administration of doses of a receptor antagonist in combination with doses of the training drug. Such responding might, but does not necessarily, indicate that the mixture is inert. Thus, the possibility exists that if the stimulus effects of the receptor antagonist in combination with the training drug or a test agent are not like those of the dose of training drug nor like the vehicle (i.e., inert) condition, then the vehicle-designated lever could serve as a default response (i.e., “transfer test over-inclusiveness” or “third-state” responding, see Section F, #2). Thus, results of complete antagonism only indicate that the stimulus effects produced by the combination of the antagonist and the training drug are dissimilar from those produced by the dose of training drug.

In instances of partial stimulus antagonism, subjects respond ~40–65% on the drug-designated lever after administration of doses of a receptor antagonist in combination with doses of the training drug. Such results indicate that drug-appropriate responding is still somewhat like the dose of training drug but also somewhat "vehicle-like"; recall, the vehicle-designated lever could be the default lever and subjects might press it under the vehicle (i.e., inert) condition or when the possible effect of the mixture of agents is dissimilar from the dose of training drug. Partial antagonism, like partial generalization, has been (and is) an ongoing topic of debate/controversy in the field.

In tests that result in no stimulus antagonism, subjects respond ≥80% on the drug-designated lever after administration of doses of a receptor antagonist in combination with doses of the training drug. Such results indicate that drug-appropriate responding is still like that of the dose of training drug and that the receptor antagonist does not interfere with the mechanisms that form the basis of the discrimination.

Stimulus antagonism can be studied by three general approaches. In one approach, doses of a receptor antagonist are combined with the dose of the training drug to determine whether the stimulus effect can be blocked. For example, rats *trained to discriminate 3.0 mg/kg of diazepam from vehicle* were administered various doses of the benzodiazepine receptor antagonist flumazenil, which reverses the effects of benzodiazepines by competitive inhibition at the benzodiazepine binding site on the $GABA_A$ receptor, prior to the administration of their dose of training drug or doses of agents that produced diazepam-like stimulus effects. If flumazenil is an effective receptor antagonist, then a dose related antagonism should occur in the animals' percentage of drug-appropriate responding. Figure 3-21 (top) shows that administration of doses of flumazenil (3.0–12 mg/kg; AD_{50} = 4.96 mg/kg [95% confidence limits 3.68–6.67 mg/kg]) prior to injections of 3.0 mg/kg (training dose) of diazepam was sufficient to produce antagonism (i.e., responding ultimately occurred on the vehicle-designated lever). In addition, injection of various doses of flumazenil (2.0–3.5 mg/kg; AD_{50} = 2.60 mg/kg [95% confidence limits 2.14–3.16 mg/kg]) prior to the administration of the dose of clordiazepoxide (5.5 mg/kg), another benzodiazepine analog, that produced complete stimulus generalization in these animals produced antagonism. In contrast, administration of doses of flumazenil (2.5–40 mg/kg) prior to the injection of the dose of pentobarbital (10 mg/kg) that produced complete stimulus generalization in these animals failed to produce antagonism (i.e., responding remained on the diazepam-designated lever). Thus, these data indicate that even though diazepam, chlordiazepoxide, and pentobarbital can exert a similar (i.e., *diazepam-like*) stimulus effect their mechanisms of action can be differentiated by flumazenil, a benzodiazepine receptor antagonist.

In comparison, another group of rats was *trained to discriminate 5.0 mg/kg of pentobarbital from vehicle* and administered various doses of flumazenil prior to the administration of their dose of training drug or doses of agents that produced pentobarbital-like stimulus effects. Figure 3-21 (bottom) shows that administration of various doses of flumazenil (1.25–40 mg/kg) before the injection of 5.0 mg/kg (training dose) of pentobarbital did not produce antagonism (i.e., responding remained on the pentobarbital-designated lever). In addition, administration of various doses of flumazenil (1.25–40 mg/kg) prior to the injection of the dose of barbital (60 mg/kg), another

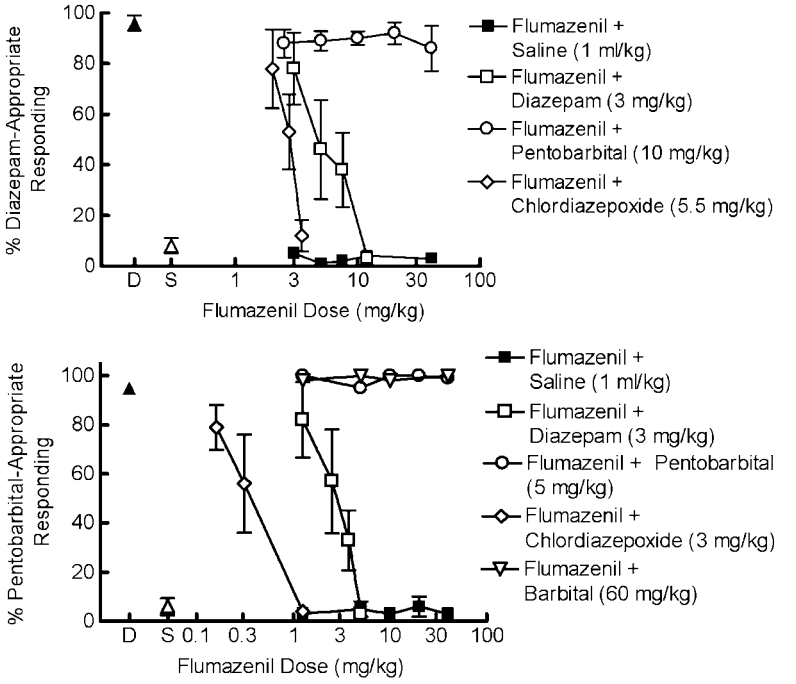

Figure 3-21. Top figure shows the effects of doses of flumazenil administered in combination with 3.0 mg/kg of diazepam (open squares), 10 mg/kg of pentobarbital (open circles), 5.5 mg/kg of chlordiazepoxide (open diamonds) or alone (solid squares) in rats trained to discriminate 3.0 mg/kg of diazepam versus vehicle. Bottom graph depicts the effects of doses of flumazenil administered in combination with 5.0 mg/kg of pentobarbital, 3.0 mg/kg of diazepam, 60 mg/kg of barbital (open inverted triangle), 3.0 mg/kg of chlordiazepoxide, or alone in rats trained to discriminate 5.0 mg/kg of pentobarbital versus vehicle. Ordinate: Mean percent (± S.E.M.) of responding on the diazepam or pentobarbital-appropriate lever after the administration of flumazenil alone or in combination with the test agents. The administration of flumazenil prior to the injection of benzodiazepines produced dose related antagonism of the training drug-like stimulus effect. In contrast, the administration of flumazenil prior to the injection of barbiturates produced no attenuation of the training drug-like response. Lastly, flumazenil, administered alone, did not induce training drug-appropriate responding. D and S represent percent responding following the training dose of diazepam (top figure) or pentobarbital (bottom graph) and saline vehicle.

barbiturate derivative, that produced complete stimulus generalization in these animals also did not produce antagonism (i.e., responding remained on the pentobarbital-designated lever). In contrast, administration of various doses of flumazenil (1.25–5.0 mg/kg; AD_{50} = 2.31 mg/kg [95% confidence limits 1.61–3.31 mg/kg]) prior to the injection of 3.0 mg/kg of diazepam (a dose that produced complete stimulus generalization in these animals) was sufficient to produce antagonism (i.e., responding occurred on the vehicle-designated lever). Similarly, injection of various doses of flumazenil

(0.15–1.25 mg/kg; AD_{50} = 0.32 mg/kg [95% confidence limits 0.19–0.54 mg/kg]) prior to the administration of the dose of clordiazepoxide (3.0 mg/kg) that produced complete stimulus generalization in these subjects produced antagonism (i.e., responding occurred on the vehicle-designated lever). Thus, these data indicate that even though diazepam, chlordiazepoxide, and pentobarbital can exert a similar (i.e., *pentobarbital-like*) stimulus effect their mechanisms of action can be differentiated by flumazenil.

Also, when each group of the above animals was administered doses of flumazenil (3.0–40 mg/kg) in control tests, they failed to respond on the diazepam- or pentobarbital-designated lever. It is noted, however, that rats, pigeons, and humans have been trained to discriminate the stimulus effects of flumazenil from vehicle and that in some (but not all) studies flumazenil stimulus generalization occurred to chlordiazepoxide and diazepam, which suggests that flumazenil can exert some degree of partial agonist action [244–247]. Nevertheless, flumazenil is a very effective antagonist in cases of benzodiazepine or zolpidem overdose [e.g., 248].

Taken together, the data indicate very clearly that even though benzodiazepine and barbiturate analogs can exert similar stimulus effects regardless of which agent is used as training drug (i.e., diazepam-like or pentobarbital-like cross-generalizations), their mechanisms of action can be differentiated by flumazenil, a benzodiazepine receptor antagonist. *More importantly, the results strongly re-emphasize the concept that stimulus generalization between a training drug and a test agent is simply evidence that both drugs can produce a similar stimulus effect but it is not necessarily accurate to conclude that they do so through an identical mechanism of action* [e.g., 74, 216].

In another example, rats trained to discriminate 1.0 mg/kg of DOM were administered various doses of the selective 5-HT_2 receptor antagonist pirenperone 45 minutes prior to the administration of their dose of training drug, or, in control studies, vehicle; 15 minutes later, stimulus control was evaluated (Figure 3-22, top figure). Pretreatment of the animals with doses of pirenperone greater than 5.0 µg/kg produced antagonism of the stimulus effects exerted by 1.0 mg/kg of DOM. Doses of pirenperone in combination with saline vehicle produced ≤5% DOM-appropriate responding; response rates (Figure 3-22, bottom figure) following the administration of pirenperone in combination with DOM or saline vehicle were consistent with those after administration of the training dose of DOM (i.e., D) or saline vehicle (i.e., S). During the antagonism tests, however, the experimenters noted that on days that followed tests with pirenperone the animals training performance was poor after the administration of the training dose of DOM. That observation led to more extensive antagonism tests that varied the PSII after pirenperone administration to determine the time course effect of antagonism of pirenperone. Such tests determined that pirenperone possessed a rather extended duration of action without marked effect on response rates (Figure 3-23, top and bottom figures). Even 6 hours after administration of 5.0 µg/kg of pirenperone, the animals performed less than 30% of their total responses on the DOM-appropriate lever 15 minutes after the administration of 1.0 mg/kg of DOM; the antagonist effects of pirenperone in combination with DOM were not evident 24 hours after the administration of the antagonist (Figure 3-23, top figure).

In a second approach to antagonism studies, the dose response of the training drug is determined in both the presence and absence of a constant dose of the antagonist. If

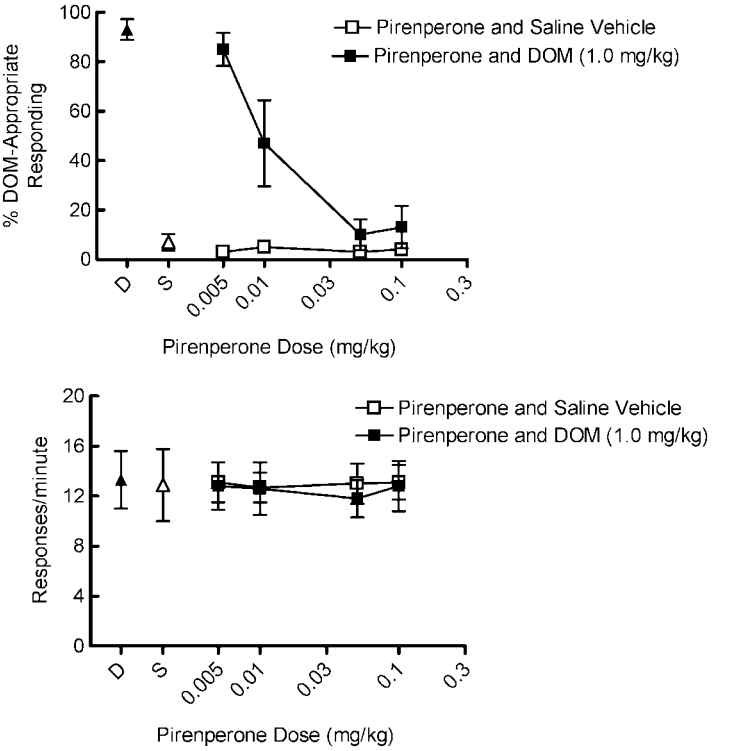

Figure 3-22. The effect of various doses of pirenperone on percent DOM-appropriate responding when administered in combination with 1.0 mg/kg of DOM (closed squares) or 1.0 ml/kg of saline (open squares). D and S represent percent DOM-appropriate responding following the training dose of 1.0 mg/kg of DOM and saline vehicle, respectively.

the antagonism is competitive, then the dose response of the training drug should shift in a rightward and parallel manner. Figure 3-24 (top figure) shows the dose response effect of diazepam in the absence (i.e., left dose response function) and the presence (right dose response effect) of flumazenil (5.0 mg/kg). As can be seen, pretreatment of the animals with flumazenil induced a rightward shift of the dose response function of diazepam.

In a third approach, various doses of the training drug can be combined with various doses of a receptor antagonist. This approach will generate a series (or family) of training-drug (agonist)/antagonist dose response curves and provide the most comprehensive or detailed picture of the interaction between the agents. Figure 3-24 (bottom figure) shows the dose response effect of diazepam in the absence (i.e., left dose response function) and the presence (middle and far right dose response effects) of flumazenil (5.0 mg/kg and 12 mg/kg, respectively). Taken together with the data in Figure 3-21, the aforementioned examples indicate that flumazenil (an imidazobenzodiazepine derivative) produced competitive antagonism, presumably at the

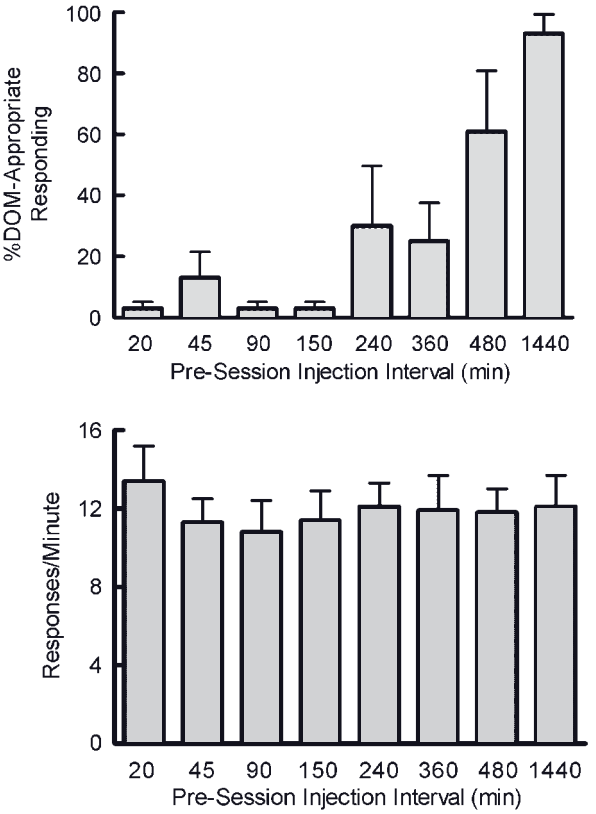

Figure 3-23. The effect of various pre-session injection intervals (PSII) 5.0 μg/kg of pirenperone administered in combination with 1.0 mg/kg of DOM. Ordinate: Data points represent group means (± S.E.M.) of percent DOM-appropriate lever responding (top figure) and response rate (bottom figure) after the administration of 5.0 μg/kg of pirenperone administered in combination with 1.0 mg/kg of DOM. See text for further discussion.

benzodiazepine recognition site on the $GABA_A$/benzodiazepine receptor complex, of the stimulus effects of benzodiazepines but not barbiturates [249, 250].

Lastly, some investigators have pursued Schild regression to quantify and characterize receptor antagonists in drug discrimination experiments. The method, originally developed for in vitro assays, aims at establishing agonist dose-effect functions with various doses of receptor antagonists. The magnitude of dose-response shifts can be expressed graphically in a Schild plot, for which ED_{50} values are determined for an agonist administered alone and in combination with at least three doses of a receptor antagonist. Schild analysis can be particularly useful for identifying or confirming the receptor(s) mediating the discriminative stimulus effects of an agonist. Agonists acting at the same receptor often have qualitatively similar discriminative stimulus effects and those effects are blocked in a predictable manner by receptor antagonists that have

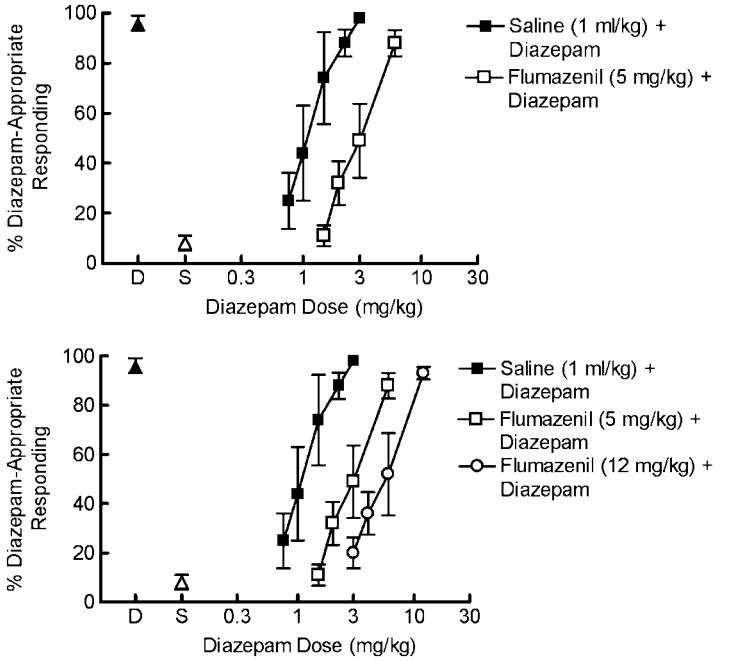

Figure 3-24. The effect of doses of diazepam alone (closed squares) or in combination with 5 mg/kg of flumazenil (open squares) in rats trained to discriminate 3.0 mg/kg of diazepam versus vehicle (top figure). The bottom figure depicts the effects of doses of diazepam alone (closed squares) or in combination with 5.0 mg/kg (open squares) or 12 mg/kg (open circles) of flumazenil. Ordinate: Data points represent group means (± S.E.M.) of % diazepam-appropriate lever responding after the administration of saline vehicle or doses of flumazenil in combination with doses of diazepam. D and S represent percent diazepam-appropriate responding following the training dose of 3.0 mg/kg of diazepam and saline vehicle, respectively.

similar apparent affinity (pA_2) values at that receptor. Examples of this approach in drug discrimination studies can be found in investigations of agonist/antagonist pairs at 5-HT_{2A} (see Chapter 13 by Li et al.), cannabinoid [251, 252], opioid (253–256], and $GABA_A$/benzodiazepine [257, 258] receptors.

G. SELECTED TOPICS

1. Sex-Related Differences

Sex differences in nonhuman animal and human reactions to a number of psychoactive agents have appeared in the scientific literature [e.g., 259–262, Chapter 15 (Perkins)]. In humans, for example, gender differences have been reported in reactions to drugs of abuse and in pharmacotherapy for depression [e.g., 263–266]. In animal studies, sex

differences in the effects of psychoactive agents have been reported in motor activity studies and assays of "emotional behavior" and spatial learning [e.g., 267–272]. Historically, drug discrimination studies—like most other types of (especially) preclinical studies—have employed mostly male subjects. That decision likely stems from a concern of the females' estrous cycle and its potential influence on her response to the training drug or test agent [e.g., 273, 274]. Predictably, the discrimination literature revealed relatively few citations that have specifically addressed and assessed possible differences in the stimulus properties of drugs in male and female subjects. Those studies are listed in Table 3-8, which also notes the training dose of drug and differences (but not similarities) in results that were observed.

From that data, some general comments on sex as a variable in discrimination studies are that 1) not many research groups have conducted discrimination studies that assessed sex as a factor; 2) not many types of agents have been examined in both sexes in the same study; cocaine, ethanol, and morphine have been studied most often; and 3) to date, there appears to be many more similarities than differences in results between

TABLE 3-8. Summary of studies that have compared drug discrimination results between female and male subjects

Training Drug (Dose)	Species	Reported Difference(s)	Reference
Cocaine (5.6 mg/kg)	Rat	Duration of action of cocaine was shorter in ♀ than in ♂	Craft & Stratmann [277]
Cocaine (10 mg/kg)	Rat	None	Anderson & Van Haaren [278]
Cocaine (10 mg/kg)	Rat	None	Anderson & Van Haaren [279]
Estradiol-17B (50 µg/kg)	Rat	None	de Beun et al. [280]
Ethanol (1.0 g/kg) Ethanol (2.0 g/kg)	Monkey	None	Grant et al. [281]
Ethanol (1.0 g/kg) Ethanol (2.0 g/kg)	Monkey	♀ but not ♂ trained to higher dose of ethanol generalized to dizocilpine, phencyclidine, or ketamine	Vivian et al. [282]
Ethanol (1.0 g/kg) Ethanol (2.0 g/kg)	Monkey	♀ trained to higher dose of ethanol not as sensitive as ♂ to allopregnanolone	Grant et al. [283]
Ethanol (1.0 g/kg) Ethanol (2.0 g/kg)	Monkey	♀ may be slightly more sensitive than ♂ to effect of gamma-hydroxybutyric acid	Helms et al. [284]
Ethanol (1.0 g/kg) Ethanol (2.0 g/kg)	Monkey	Generalization of Ro15-4513[a] was more potent in ♂ than in ♀	Helms et al. [285]
LHRH[b] (5.0 µg/kg)	Rat	♂ but not ♀ learned discrimination	de Beun et al. [286]
mCPP[c] (1.2 mg/kg)	Rat	♀ less sensitive than ♂ to mCPP	Jung et al. [287]

TABLE 3-8. (*Continued*)

Training Drug (Dose)	Species	Reported Difference(s)	Reference
Morphine (3.0 mg/kg)	Rat	♀ learned DD faster than ♂; generalization of morphine and buprenorphine was more potent in ♀ than in ♂	Craft et al. [288]
Morphine (3.0 mg/kg)	Rat	None	Craft et al. [289]
Morphine (3.0 mg/kg)	Rat	Generalization of morphine was more potent in ♀ than in ♂	Craft et al. [290]
Morphine (3.0 mg/kg)	Rat	Generalization of morphine was more potent in ♀ than ♂ under a FR but not a VI schedule of reinforcement	Krivsky et al. [291]
(–)Nicotine (12 μg/kg)	Human	Subjective reports	Perkins et al. [292]
(–)Nicotine (10 μg/kg) (–)Nicotine (30 μg/kg)	Human	♀ in lower training dose group of nicotine were more sensitive to a lower dose of nicotine than ♂	Perkins et al. [293]
(–)Nicotine (20 μg/kg)	Human	♀ but not ♂ may exhibit acute tolerance to nicotine	Perkins et al. [294]
Pentobarbital (12 mg/kg)	Rat	Ovariectomized ♀ but not (intact) ♂ generalized to progesterone	Heinsbroek et al. [295]
Pentylenetetrazol (16 mg/kg)	Rat	♀ less sensitive than ♂ to pentylenetetrazol to pentylenetetrazol	Jung et al. [296]
Pregnanolone (10 mg/kg)	Mice[d]	None	Shannon et al. [297]
S(+)amphetamine (15 mg)	Human	Subjective reports	Vansickel et al. [298]
U-69,593[e] (0.13 mg/kg)	Rat	♂ learned discrimination faster than ♀; Peak effect and duration of action were faster in ♀ than in ♂; generalization of U-69,593 and bremazocine was more potently in ♂ than in ♀ at start of study (equi-potent at end of study)	Craft et al. [299]

Table includes dose of training drug, species, and reported difference in results.
[a]Ro15-4513 (Ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-1,4-benzodiazepine-3-carboxylate).
[b]LHRH (luteinizing-hormone releasing hormone) also known as gonadotropin-releasing hormone (GnRH).
[c]meta-Chlorophenylpiperazine.
[d]Males and females of two strains of mice: DBA/2J and C57BL/6J.
[e]U-69,593 (5α,7α,8β)-(–)-*N*-Methyl-[7-(1-pyrrolidinyl)-1-oxaspiro(4,5)dec-8-yl]benzeneacetamide.

the sexes in the stimulus effects of drugs. However, some drug discrimination studies have demonstrated, to various degrees, gender-related results (Table 3-8). In brief, those sex-dependent results seem to be concentrated on the rate at which, or the demonstration that, a particular sex learned the discriminative stimulus effect of a drug, how potent a training drug or test agent was in a group, and/or the duration of action of a drug. Such differential action may be related to sex-related differences in the pharmacokinetic, pharmacodynamic, and/or behavioral pharmacology of a drug. Thus, an agent may exert reactions that are sex-related because of differences in its transport within the body, metabolism by enzymes, and/or mechanism of action [275, 276]. Future drug discrimination studies in which sex is assessed as a factor are likely to increase, especially if (human) reports continue to surface of gender differences in response to drugs, particularly centrally active agents.

2. Selected Line and Inbred Strains of Animals

Selected lines and inbred strains of rodents have been employed to examine the potential influence that genetics may exert in the discriminative stimulus effects of drugs (Table 3-9). Historically, such animals preceded the present innovations in molecular biology (see below), which require more sophisticated biological techniques and methods. All of the models, however, are based on the intent to manipulate animals' genetic material to study the potential effects on a behavior of interest. In one approach, an animal species may be bred selectively to enhance a desired characteristic (i.e., phenotype). Moreover, the phenotype may be selected for both high and low levels (i.e., bi-directional selection) and its progressive separation (divergence) should be observed throughout generations of the animals until stable differences are noted. Thus, selected lines are created when unrelated animals with similar characteristics are mated (i.e., crossed). In such cases, the genes of the two "lines" that underlie the phenotype are assumed to be different; genes that underlie other traits are thought to remain similar. Researchers have created selected lines of animals that have extremely high or extremely low levels of an attribute, behavior, or phenotype of interest. For example, investigators have bred rats that differ in their sensitivity or voluntary preference for the consumption of alcohol; "alcohol preferring versus nonpreferring," "high alcohol-drinking versus low alcohol-drinking," and "high alcohol-sensitive versus low alcohol-sensitive." The rationale for the use of such animals stems from assessments in humans of an inherited component of responses to alcohol. For example, twin and adoption studies have suggested a genetic component of vulnerability to alcoholism [e.g., 300].

In comparison, inbred strains are generated when male and female siblings are mated, regardless of any particular trait, over several generations. Such strains are termed inbred if they have been mated brothers by sisters for ≥20 consecutive generations, and individuals of the strain can be traced to a single ancestral pair at the 20th or subsequent generation. The result of this process is a population of animals in which only one allele of every gene is present, which is homozygous for every allele. Consequently, all animals within an inbred strain are considered genetically "identical." Thus, researchers employ these populations for study because the animals' genetic makeup is believed to have been "fixed," except for changes that might result from

TABLE 3-9. Studies of drug discrimination that compared results (i.e., percentage drug-lever appropriate responses) between selected lines and inbred strains

Training Drug (dose)	Strain Comparison[a]	Reported Difference(s)	Reference
Clozapine (2.5 mg/kg)	♂ C57BL/6J vs. DBA/2J mice	C57BL/6J generalized fully and DBA/2J generalized partially to ziprasidone; C57BL/6J but not DBA/2J generalized to ritanserin and prazosin	Porter et al. [302]
Cocaine (10 mg/kg)	♂ Lewis vs. Fischer 344 rats	None	Haile et al. [303]
Cocaine (10 mg/kg)	♂ Lewis vs. Fischer 344 rats	None	Haile & Kosten [304]
Dizocilpine (0.17 mg/kg)	C57BL/6J vs. DBA/2J mice	C57BL/6J generalized fully and DBA/2J generalized partially to phencyclidine	Shelton & Balster [305]
Ethanol (1.0 g/kg) followed by 2.0 g/kg for C3H/HeNCr	♂ C57BL/6J vs. C3H/HeNCr mice	C57BL/6J acquired and maintained ethanol discrimination better than C3H/HeNCr	Becker et al. [306]
Ethanol (1.5 g/kg)			
Ethanol (1.5 g/kg)	♂ C57BL/6J vs. DBA/2J mice	DBA/2J learned ethanol discrimination faster than C57BL/6J; generalization of pentobarbital was more potent in DBA/2J than in C57BL/6J	Shelton & Grant [307]
Ethanol (0.5 g/kg)	High alcohol-drinking vs. Low alcohol-drinking rats	None	McMillan & Li [308]
Ethanol (0.75 g/kg vs. 1.5 g/kg vs. saline)	♂ High alcohol-drinking vs. Low alcohol-drinking rats	Low alcohol-drinking learned 3-key ethanol discrimination faster than high alcohol-drinking	McMillan & Li [309]
Ethanol (0.6 g/kg)	♂ High alcohol-sensitive vs. Low alcohol-sensitive rats	None	Krimmer [310]
Ethanol (1.0 g/kg)	♂ High alcohol-sensitive vs. Low alcohol-sensitive rats	None	Krimmer [311]

(*Continued*)

TABLE 3-9. (*Continued*)

Training Drug (dose)	Strain Comparison[a]	Reported Difference(s)	Reference
Ethanol (0.75 g/kg)	♂ High alcohol-drinking vs. Low alcohol-drinking rats	None	Krimmer [312]
Ethanol (0.5 g/kg)	♂ High alcohol-drinking vs. Low alcohol-drinking rats	None	Krimmer & Schechter [313]
Ethanol (1.0 g/kg)	♂ Alcohol-preferring vs. Alcohol-nonpreferring rats	Alcohol-preferring but not Alcohol-nonpreferring generalized partially to (–) nicotine	Gordon et al. [314]
Ethanol (0.6 g/kg)	♂ Sprague-Dawley vs. Fawn-Hooded vs. N/Nih Rats	Sprague-Dawley and N/Nih learned ethanol discrimination faster than Fawn-Hooded	Schechter & Meehan [315]
Ethanol (0.6 g/kg)	♂ High alcohol-drinking vs. Low alcohol-drinking rats	High alcohol-drinking but not Low alcohol-drinking generalized to MDMA[b]	Meehan et al. [316]
Ethanol (0.6 g/kg) for N/Nih Ethanol (1.0 g/kg) for Fawn-Hooded	Fawn-Hooded vs. N/Nih rats	Generalization of ethanol was more potent in N/Nih than in Fawn-Hooded	Schechter et al. [317]
Ethanol (1.0 g/kg)	♀ Alcohol-accepting vs. Alcohol-nonaccepting rats	Alcohol-nonaccepting acquired and maintainedethanol discrimination better than Alcohol-accepting	York [318]
Ethanol (780 mg/kg)	♀ Alcohol-preferring vs. Alcohol-nonpreferring rats	None	York [319]
Ethanol (0.5 g/kg) (–) Nicotine (0.6 mg/kg)	Alcohol-preferring vs. Alcohol-nonpreferring rats	Alcohol-preferring learned ethanol discrimination faster than Alcohol-nonpreferring; Alcohol-preferring, but not Alcohol-nonpreferring, generalized fully to nicotine and partially to *S*(+)-amphetamine; Alcohol-nonpreferring nearly generalized and Alcohol-preferring generalized partially to bupropion	McMillan et al. [320]

MDMA[b] (1.5 mg/kg)	♂ Fawn-Hooded vs. Sprague-Dawley rats	None	Schechter [321]
Morphine (3.0 mg/kg) Morphine (5.6 mg/kg)	Lewis vs. Long Evans vs. Sprague-Dawley vs. Fischer F 344 rats	None	Morgan et al. [322]
(–)Nicotine (0.4 mg/kg) for Lewis (–)Nicotine (0.9 mg/kg) For Fischer 344	♂ Lewis vs. Fischer 344 rats	Lewis rats learned discrimination at lower dose of nicotine than Fischer 344	Philibin et al. [323]
(–)Nicotine (0.4 mg/kg) (–)Nicotine (0.8 mg/kg) (–)Nicotine (1.6 mg/kg) for C57BL/6J (–)Nicotine (0.8 mg/kg) for DBA/2J	♂ C57BL/6J vs. DBA/2J mice	None	Stolerman et al. [324]
Pregnanolone (10 mg/kg)	♂ and ♀ C57BL/6J vs. ♂ and ♀ DBA/2J mice	Generalization of pentobarbital or midazolam was more potent in DBA/2J than in C57BL/6J	Shannon et al. [297]
Pregnanolone (5.6 mg/kg)	♂ C57BL/6J vs. ♂ DBA/2J mice	DBA/2J but not C57BL/6J generalized to NMDA receptor antagonists; generalization of AlloTHDOC[c] and midazolam was more potent in DBA/2J than in C57BL/6J	Shannon et al. [325]

[a]If sex of rodent is not indicated, then it was not stated in reference.
[b]*N*-Methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane.
[c]Allotetrahydrodeoxycorticosterone.

spontaneous alterations (i.e., mutations) in a particular animal's genetic material. In drug discrimination experiments, researchers compare inbred strains of animals in the acquisition of the drug discrimination task and/or results from tests of stimulus generalization/antagonism. If differences are noted between different inbred strains that have the same environmental history, then those differences are attributed to genetic factors. As noted previously (see Chapter 1, Subjects), inbred rats such as Lewis (LEW) and Fischer 344 (F 344) are reported to be differentially sensitive to drugs of abuse and situations of stress. Inbred strains of mice such as C57BL/6J and DBA/2J also display differences in biology and behavior [e.g., 301].

The drug discrimination literature related to selected lines and inbred strains of rodents revealed a number of citations that have specifically addressed and assessed possible differences in the stimulus properties of drugs. Those studies are listed in Table 3-9, which also notes the training dose of training drug, inbred strain comparisons, and reported differences (but not similarities) in results that were observed. From that data, some general comments on the results are that 1) ethanol and (–)nicotine have been studied most often as stimuli followed by cocaine and morphine; 2) an approximately equal number of studies have reported some degree of difference versus no difference in results between strains of animals; and 3) when differences between strains are noted they relate to the rate or speed at which a discrimination was learned, differences in how potent a training drug was in a strain or the degree of stimulus generalization of the training drug to test agents.

3. Transgenic and Knockout Strains

In comparison to selected lines and inbred strains of animals, two other, more specific, genetic approaches have been employed to assess the function of genes on behavior: transgenic and knockout mice. A review of the literature, however, revealed that the use of such mice in drug discrimination tasks appears to have just begun and, consequently, is limited to just a few studies (Table 3-10). In transgenic mice, a foreign gene is integrated into the animals' genetic material, which allows investigators to study the effects of that gene alteration on behavior. For example, CNS receptors linked to serotonin may exert a role, at least in part, in an organism's consumption of ethanol and behaviors that are related to ethanol [e.g., 326]. In a test of this idea, transgenic mice were created with "over expressed" serotonin 5-HT_3 receptors and found to exhibit increased (initial) sensitivity to low doses (e.g., 1.5 g/kg) of ethanol; that is, transgenic mice consumed less ethanol than control mice [327, 328]. In a drug discrimination study, however, transgenic mice with over-expressed 5-HT_3 receptors and control animals were trained to discriminate the stimulus effects of 1.5 g/kg of ethanol from vehicle but no differences were observed in their acquisition of the task or in stimulus generalization/antagonism tests [329]. In knockout (KO) mice, a gene of interest is inactivated and conclusions are drawn about the function of that gene by the consequences of its absence. Drug discrimination studies that compared KO versus control mice are listed in Table 3-10, which also notes the training dose of training drug, receptor that was inactivated, and reported differences (but not similarities) in results that were observed. When differences were noted, they appeared to be related to the rate at

TABLE 3-10. Summary of studies that have compared results of DD studies in transgenic, knockout, and control mice

Training Drug (Dose) versus Vehicle	Transgenic Mice	Reported Difference(s)	Reference
Cocaine (10 mg/kg)	♂ and ♀ Dopamine D_2 KO vs. heterozygous (HET) vs. wild-type	Raclopride produced antagonism of cocaine in wild-type and HET but not in KO	Chausmer et al. [339]
Cocaine (10 mg/kg)	♂ and ♀ Dopamine D_5 KO vs. heterozygous(HET) vs. wild-type	None	Elliot et al. [340]
Cocaine (10 mg/kg)	♂ Dopamine D_4 KO vs. wild-type	Generalization of cocaine was more potent in KO than in wild-type; raclopride was more potent antagonist of cocaine in KO than in wild-type.	Katz et al. [341]
Ethanol (1.5 g/kg)	$GABA_A$ δ subunit KO vs. wild-type	KO generalized fully and wild-type generalized partially to pentobarbital	Shannon et al. [342]
Ethanol (1.5 g/kg)	Serotonin $5\text{-}HT_3$ over-expressed vs. wild-type	None	Shelton et al. [329]
LSD[a] (0.17 mg/kg) followed by LSD (0.3 mg/kg) followed by Pentobarbital (15 mg/kg) followed by Pentobarbital (30 mg/kg)	Serotonin transporter KO vs. wild-type	Wild-type, but very few KO, learned LSD discrimination; wild-type, but only one-half of KO, learned pentobarbital discrimination	Krall et al. [343]
(–)Nicotine (0.4 mg/kg) (–)Nicotine (0.8 mg/kg)	♂ Nicotinic α7 subunit KO vs. wild-type	None	Stolerman et al. [344]
(–)Nicotine (0.4 mg/kg) (–)Nicotine (0.8 mg/kg) (–)Nicotine (1.6 mg/kg)	♂ Nicotinic β2 subunit KO vs. wild-type	Wild-type learned discrimination at each dose of (–)nicotine; KO learned discrimination only at highest dose of (–)nicotine	Shoaib et al. [345]

Data obtained from citations in Drug Discrimination bibliography (http://www.drugref.org).
[a](+)Lysergic acid diethylamide.

which, or the demonstration that, a particular KO strain learned the discriminative stimulus effect of a drug and/or how potent a training drug or test agent was in a group. In the future, transgenic and KO mice will likely become more frequent subjects in drug discrimination studies because of (an expected) increase in the availability of many types of strains, such as KOs for dopamine$_{D1}$ [e.g., 330], GABA$_{\alpha 6,\beta 3, \text{ or } \gamma 2L}$ subunits (e.g., 331–333], 5-HT$_{1B}$ [e.g., 334–336], 5-HT$_{2C}$ [337], and dopamine transporter [e.g., 338] receptors.

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4

ROLE OF STEREOCHEMISTRY IN DRUG DISCRIMINATION STUDIES

A. STRUCTURAL ISOMERS: INTRODUCTION

Drug discrimination (as well as other pharmacological and biochemical) studies frequently employ "*optically active*" or "*chiral*" agents, but investigators sometimes neglect to explicitly state this basic fact in their publications, or fail to appreciate the

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

ramifications. For example, when "amphetamine" is used as a training drug or test drug, it can only (i.e., it *must*) be assumed that (±)- or "*racemic*" amphetamine is being employed unless otherwise stated. The stereochemistry (i.e., the structural isomers) of training drugs and test drugs has a profound, and frequently underappreciated, effect on the results of pharmacological studies. *Structural isomers are chemical entities with identical empirical formulas that differ in the nature or sequence of their atoms*. These isomers can also differ substantially with regard to the pharmacological effects they produce. For example, the desired action of an agent may rest with one of two or more isomers and the other(s) serve only as "filler" to dilute the actions of the racemic (or diastereomeric) mixture. Two broad categories of structural isomers include *constitutional isomers* and *stereoisomers*. Both types of isomers are frequently encountered in drug discrimination studies. For much greater detail on stereochemistry and general stereochemical principles, readers are referred to *Basic Organic Stereochemistry* [1]. For a discussion of the impact of stereochemistry on the psychopharmacological actions of centrally acting agents, see *Handbook of Stereoisomers: Drugs in Psychopharmacology* [2].

B. CONSTITUTIONAL ISOMERS

Constitutional isomers are chemical compounds that differ with regard to their *constitution*, such as in the location of one or more atoms [1]. *Tautomers*, one specific type of constitutional isomer, differ with regard to the placement of a hydrogen atom (these will not be discussed here, but can be important in biochemical studies). Constitutional isomers also include "*positional isomers*" (also known as "*regioisomers*"); they represent a particular type of constitutional isomer frequently encountered in pharmacological (and drug discrimination) studies. Positional isomers may, but most often *do not*, produce similar pharmacological actions. Positional isomers are very commonly examined in drug discrimination studies. Examples will be provided for purpose of illustration.

There are three possible ring-monomethylated analogs (i.e., positional isomers or regioisomers) of the phenylisopropylamine psychostimulant amphetamine—referred to as toylaminopropanes (TAPs) (see Figure 4-1 for chemical structures). These are positional isomers in that a methyl (i.e., -CH_3) group can be situated at the ring 2- (or *ortho*) position, 3- (or *meta*) position, or 4- (or *para*) position, and are commonly referred to as *o*TAP, *m*TAP, and *p*TAP, respectively. They have the same empirical formula ($C_{10}H_{15}N$ for the free base) but differ in their constitution (i.e., the location of the methyl group).

Using rats trained to discriminate 1.0 mg/kg of (+)amphetamine from saline vehicle in a two-lever operant procedure, the amphetamine stimulus generalized to *o*TAP (ED_{50} = 4.1 mg/kg), but not to *m*TAP or *p*TAP [3]. Administration of doses of the latter two positional isomers of TAP resulted only in partial generalization at the doses evaluated, and in behavioral disruption when examined at higher doses [3]. That is, only *o*TAP produced stimulus effects similar to those produced by (+)amphetamine and, in this respect, *o*TAP was 10-fold less potent than the training drug. The other two isomers produced a pharmacological effect that disrupted the animals' behavior.

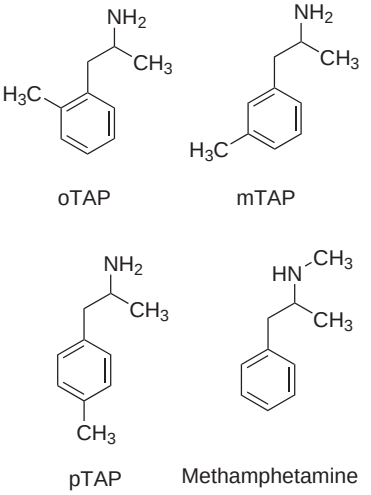


Figure 4-1. Chemical structures of four monomethylated positional isomers of amphetamine (note the position of the methyl, or $-CH_3$, group).

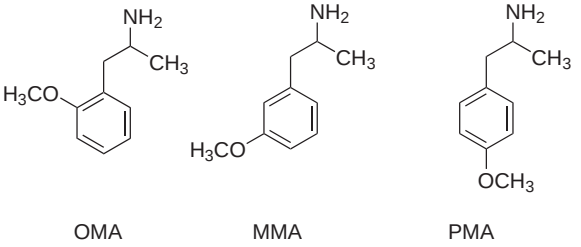


Figure 4-2. Positional isomers of (aryl-)monomethoxy-substituted amphetamine: OMA, MMA, and PMA.

Methamphetamine is another example of a positional isomer of the TAPs where an aryl-associated methyl group has been transposed from the *aryl group* (or aromatic ring) to the terminal amine. Racemic methamphetamine is more potent (ED_{50} = 0.7 mg/kg) than *o*TAP in rats trained to discriminate (+)amphetamine (1.0 mg/kg) from vehicle. Hence, only two of these four positional isomers (or regioisomers) produced amphetamine-like stimulus effects, and the two that did differed in potency by about 6-fold.

OMA, MMA, and PMA (see Figure 4-2 for chemical structures) are the three monomethoxy analogs of amphetamine (i.e., the *ortho*-, *meta*-, and *para*-methoxy analogs, respectively); they also are positional isomers. A (+)amphetamine stimulus generalized to all three positional isomers with the following order of potency: PMA (ED_{50} = 1.9 mg/kg) > MMA (ED_{50} = 3.4 mg/kg) > OMA (ED_{50} = 7.8 mg/kg) [4]. In this instance, all three positional isomers were active. Note: There is no need to report results here in μmole/kg because all three agents possess the same molecular weight.

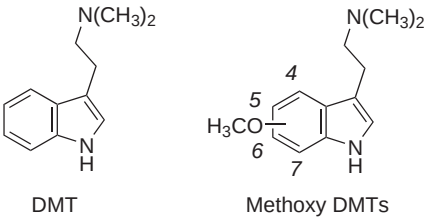


Figure 4-3. Chemical structures of N,N-dimethyltryptamine (DMT) and its four possible monomethoxy positional isomers 4-OMe DMT, 5-OMe DMT, 6-OMe DMT, and 7-OMe DMT.

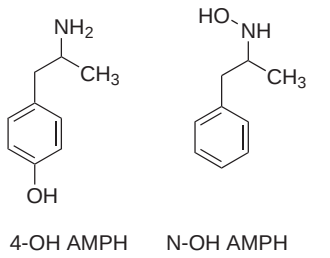


Figure 4-4. Chemical structures of two metabolites of amphetamine: 4-hydroxyamphetamine (4-OH AMPH) and N-hydroxyamphetamine (N-OH AMPH).

N,*N*-Dimethyltryptamine (DMT; see Figure 4-3 for chemical structure) is a naturally occurring hallucinogenic agent. Four aryl-substituted methoxy derivatives are possible, known as 4-methoxy-*N*,*N*-dimethyltryptamine (4-OMe DMT), and its positional isomers 5-OMe DMT, 6-OMe DMT, and 7-OMe DMT that differ structurally depending upon the location of the methoxy group. Using rats trained to discriminate 1.5 mg/kg of 5-OMe DMT, stimulus generalization occurred to all compounds shown in Figure 4-3 with the following rank order of potency (mg/kg; μmoles/kg): 5-OMe DMT (0.40; 1.3) > DMT (0.74; 2.66) > 4-OMe DMT (1.07; 3.47) > 6-OMe DMT (1.56; 5.06) ≈ 7-OMe DMT (1.51; 5.92) [5]. Here, too, all four monomethoxy positional isomers were active.

In addition to being positional isomers, 4-hydroxyamphetamine (4-OH AMPH) and *N*-hydroxyamphetamine (*N*-OH AMPH) (Figure 4-4) are metabolites of amphetamine. They differ with respect to the position of a hydroxyl group. Does either hydroxylated metabolite of amphetamine retain amphetamine-like stimulus action? Racemic *N*-OH AMPH (ED_{50} = 0.38 mg/kg) was found to be at least as potent as racemic amphetamine (ED_{50} = 0.42 mg/kg), whereas racemic 4-OH AMPH produced saline-appropriate responding at doses of up to 10 mg/kg [4, 6; also see Chapter 3]. In this case, one positional isomer produced amphetamine-like effects and one did not. Frequently, positional isomers of a given agent fail to produce a common stimulus effect.

Other examples of positional isomers include agents related to the hallucinogen DOM (Figure 4-5). For example, using rats trained to discriminate 1.0 mg/kg of DOM

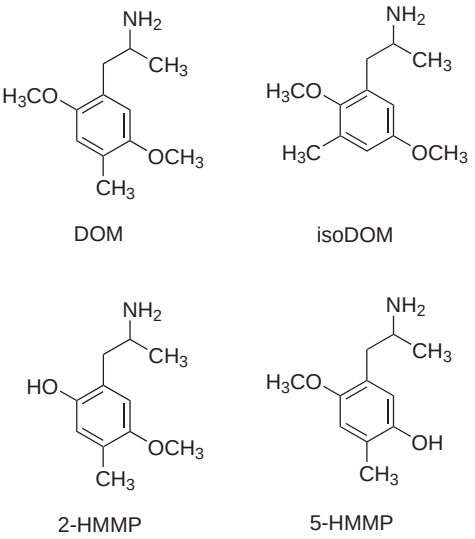


Figure 4-5. Chemical structures of the hallucinogen DOM, its positional isomer isoDOM, its 2-desmethyl analog 2-HMMP (also known as 2-DM-DOM) and its positional isomer 5-HMMP (also known as 5-DM-DOM).

from saline vehicle, DOM stimulus generalization failed to occur to a positional isomer, isoDOM, following administration of doses of up to 20 times the ED_{50} dose of DOM (i.e., 0.44 mg/kg) [7]. Examination of 2-HMMP, the 2-*des*methyl metabolite of DOM (also called 2-DM DOM), and 5-HMMP, the 5-*des*methyl metabolite (also called 5-DM DOM) (Figure 4-5), a positional isomer of 2-HMMP, showed that DOM-stimulus generalization occurred to 2-HMMP (ED_{50} = 1.71 mg/kg), but that administration of low doses of 5-HMMP produced only saline-like responding followed, at a higher dose, by disruption of the animals' behavior [7]. With a different training drug [i.e., (+)LSD], pre-session injection interval, and strain of rat (Fischer 344 rather than Sprague-Dawley), the (+)LSD stimulus generalized to both metabolites with the 5-desmethyl metabolite 5-HMMP being approximately twice as potent as its positional isomer [8].

What should be rather apparent from the above examples is that, *in the absence of other information*, it is virtually impossible to predict, *a priori*, the stimulus (or any other pharmacological) character or potencies of positional isomers. The one area where examination of positional isomers has found broad application, and has proven to be of substantial benefit, is for the formulation of *structure–activity relationships* (SAR; see Chapter 5).

Consider again the serotonergic hallucinogen 5-methoxy-*N*,*N*-dimethyltryptamine (5-OMe DMT). In the course of a structure-activity study to address the importance of the indolic N_1-position nitrogen atom to activity, two different types of positional isomers were prepared and examined [9]. First, 6-OMe DMT is a positional isomer of 5-OMe DMT (see Figure 4-6). The isotryptamine 5-OMe isoDMT is also a positional

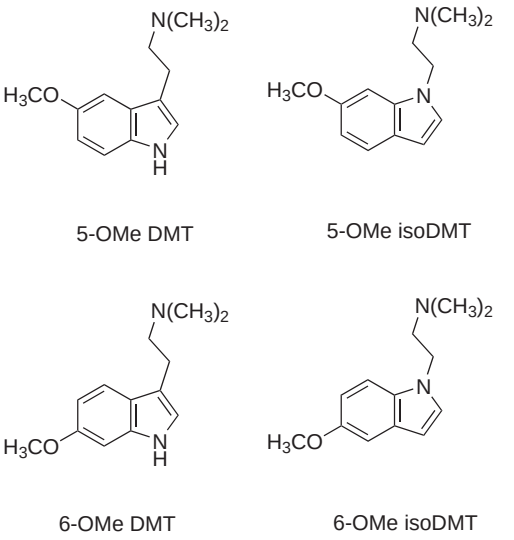


Figure 4-6. Chemical structures of a series of related positional isomers.

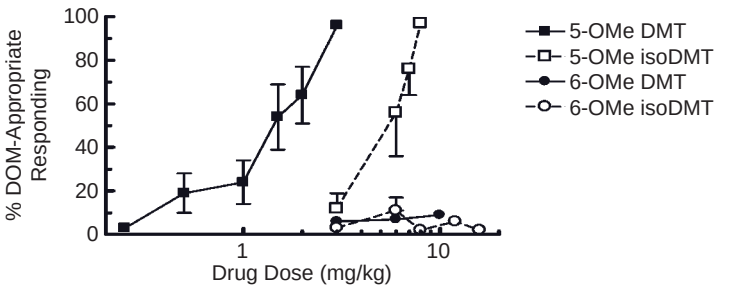


Figure 4-7. Results of stimulus generalization studies with agents shown in Figure 4-6 using rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle [9]. Administration of 1.0 mg/kg of DOM resulted in >90% drug-appropriate responding whereas administration of saline elicited <20% drug-appropriate responding (data not shown).

isomer of 5-OMe DMT. 6-OMe isoDMT is a positional isomer both of 6-OMe DMT and 5-OMe isoDMT. In this manner, it was determined that the N_1-nitrogen atom contributes to potency. That is, a DOM stimulus generalized to 5-OMe DMT (ED_{50} = 1.2 mg/kg) and 5-OMe isoDMT (ED_{50} = 7.1 mg/kg) but not to 6-OMe DMT (which produced saline-appropriate responding up to a dose of 10 mg/kg) and 6-OMe isoDMT (which produced saline-appropriate responding up to a dose of 16 mg/kg and disruption of behavior at 20 mg/kg) (Figure 4-7). In addition, the duration of action of 5-OMe DMT and 5-OMe isoDMT were identical. Hence, the presence of the N_1-nitrogen atom had an influence on potency, but did not influence stimulus generalization character or duration of action [9].

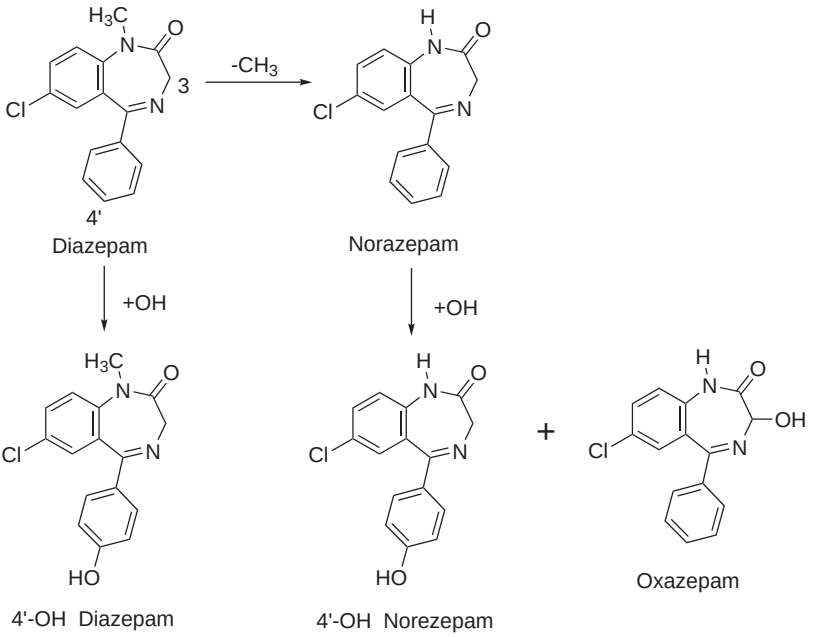


Figure 4-8. Selected routes of metabolism of the anxiolytic agent diazepam via hydroxylation, or demethylation followed by hydroxylation.

Figure 4-8 shows the hepatic metabolism of the anxiolytic (i.e., antianxiety) agent diazepam. Diazepam is metabolized by hydroxylation at the 4'-position to afford 4'-OH diazepam, and is also demethylated to norazepam. Norazepam is subsequently hydroxylated at the 4'-position to afford 4'-OH norazepam and at the 3-position to form oxazepam. At the time the studies were conducted, the "diazepam-like" stimulus actions of these metabolites were unknown; but, it was certainly of interest to determine if the diazepam metabolites contributed to the stimulus actions of diazepam. Using rats trained to discriminate 3.0 mg/kg of diazepam from saline vehicle, it was readily possible to examine each of the metabolites [10; also see Chapter 3]. It might be noted that 4'-OH norazepam and oxazepam are *positional isomers* (i.e., they differ only by the location of their hydroxyl group; that is, they possess the same *constitution* or empirical formula).

As shown in Figure 4-9 [10], the diazepam metabolite oxazepam was nearly equipotent with diazepam in producing diazepam-like stimulus effects. On the other hand, norazepam was several-fold less potent than diazepam, and the two 4'-hydroxy metabolites were at least 20-fold less potent. In fact, neither 4'-OH metabolite (i.e., 4'-hydroxydiazepam and 4'-hydroxynorazepam) produced diazepam-like stimulus effects even at >20 times the ED_{50} dose of diazepam (i.e., they produced saline-appropriate responding). The results suggested that 4'-OH metabolites of diazepam do not contribute to diazepam-like stimulus actions. It might be noted that oxazepam (currently marketed as "Serax"®) is clinically available as a shorter-acting form of

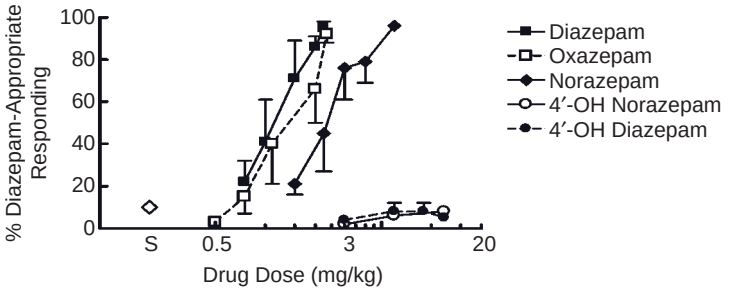


Figure 4-9. Results of stimulus generalization studies with diazepam metabolites in rats trained to discriminate 3.0 mg/kg of the anxiolytic agent diazepam from saline vehicle. S = effect of saline (1 ml/kg).

the anxiolytic agent diazepam because it is more readily conjugated and excreted than diazepam.

Here is an instance (which is more frequently the case than not) where positional isomers failed to produce a common stimulus effect. Indeed, it is rather uncommon for *positional isomers* to produce a similar stimulus effect in animals.

C. STEREOISOMERS

Stereoisomers are structural isomers with the same constitution but that differ in spatial arrangement [1]. Major categories include 1) *conformational isomers*, 2) *configurational isomers*, and 3) *optical isomers*.

1. Conformational Isomers

Conformational isomers ("*conformers*") are stereoisomers that are separated by relatively low energy barriers that can be interconverted by rotation about single bonds. Not all conformational isomers are of equivalent energy. The most classical example in chemistry is that of the *chair-boat* conformations of cyclohexane [1]. As shown in Figure 4-10, the R-substituted cyclohexane (Figure 4-10A) can exist with its R group being in either an *axial* or *equatorial* position. With monosubstituted compounds, the R group is typically equatorial because this is a lower energy conformation (i.e., there is less steric hindrance between substituents) than if the R group was axial. Figure 4-10C shows that the R_e structure (*chair* conformation) exists in equilibrium with its higher-energy *boat* form, and that the boat form exists in equilibrium with the *inverted chair* conformation where R is axial. For this compound, the chair form predominates where R = equatorial.

With di-substituted compounds, the situation becomes more complicated. The size (i.e., steric bulk) of ring substituents can dictate relative conformation. Also, 1,3- and 1,4-interactions (i.e., interactions between a substituent at the 1-position with those at the ring 3- or 4-position), such as the relative size of individual substituents, steric

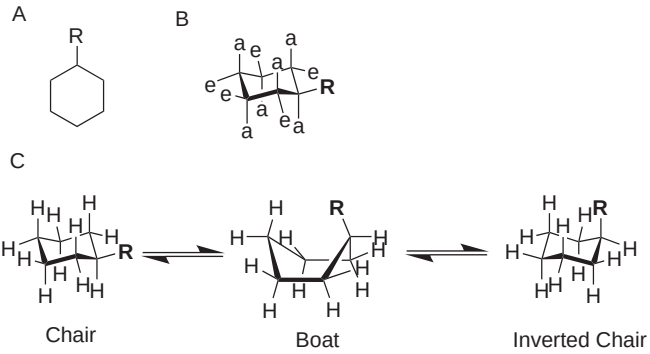


Figure 4-10. A substituted cyclohexane (A) drawn in a chair conformation showing the equatorial (e) and axial (a) nature of substituents (B). Also shown is the interconversion between chair and boat conformations (C).

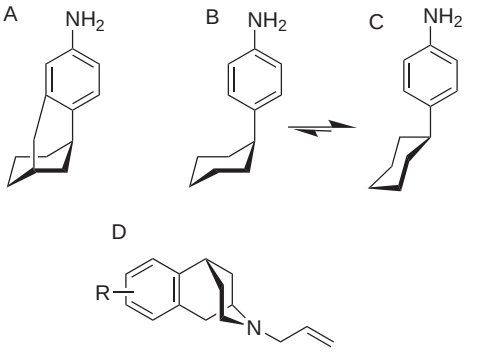


Figure 4-11. Cyclic structures and conformational isomerism.

repulsion between substituent groups, or attraction involving hydrogen bond formation, for example, can disfavor or favor particular conformations. A related example involves ring-opening of di-substituted compounds. For example, Figure 4-11A shows a conformationally constrained compound where the phenyl group is "locked" into the axial position by virtue of being incorporated into a fairly rigid ring structure. In the course of an SAR study, it might be of interest to investigate the role of the "methylene bridge" that connects the substituted phenyl group to the cyclohexyl portion of the molecule by eliminating the bridge to afford the "unlocked" structure (Figure 4-11B). However, due to the (normally) lower energy of equatorially-substituted compounds over axially-substituted [1], and given the equilibrium shown in Figure 4-10C, the target will very likely exist as shown in Figure 4-11C. There is the possibility, then, that a "ring-opened" target might not closely resemble the conformation found in the more constrained parent. These were factors to be considered when the discriminative stimulus and other pharmacological properties of, for example, *N*-allylmormetazocine analogs (Figure 4-11D) were examined [11].

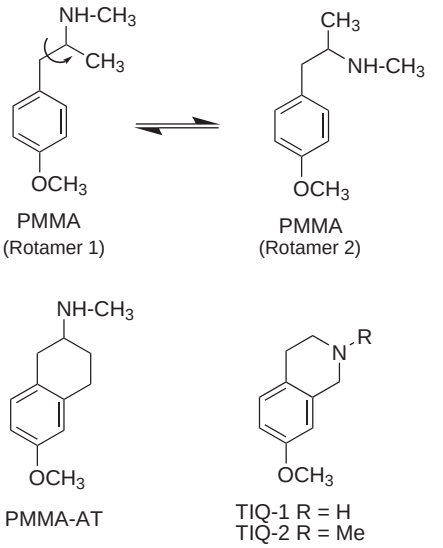


Figure 4-12. Chemical structures of PMMA and some conformationally constrained analogs.

a. Rotamers Rotamers are a special case of *conformational isomers*. Any one of several rotamers might be equally stable (i.e., equi-energetic), but a biological system (e.g., receptor) might "prefer" or accommodate one rotamer over another.

b. Conformationally Constrained Analogs The hypothesis that a biological system might prefer one rotamer over another can be tested by preparing conformationally constrained forms of the agent that "lock" the molecule into one of several different rotamers [12]. For example, PMMA (Figure 4-12) is an agent that has been found on the clandestine market, and has been used as a training drug in drug discrimination studies [13]. PMMA is a conformationally flexible molecule, and its preferred conformation for eliciting stimulus effects was unknown. Many different rotamers are possible (not all necessarily being energetically equivalent); two possible rotamers are shown. Figure 4-12 shows structures that essentially constrain or "lock" the side chain into two extreme conformations (i.e., an aminotetralin conformation as with PMMA-AT that mimics the structure of Rotamer 1, and a tetrahydroisoquinoline conformation represented by TIQ-1 and TIQ-2 that mimic the structure of Rotamer 2). Neither might be a preferred conformation. That is, the preferred conformation might be that where there is a *gauche* relationship between the side chain and the aromatic ring of PMMA. Another caveat is that conformational constraint can add additional atoms to a molecule, and the added atoms might either contribute to, or detract from, the desired action (e.g., the atoms may or may not be tolerated by a receptor). Nevertheless, examination of these "rigid" agents might provide useful information.

Following administration to rats trained to discriminate 1.25 mg/kg of (±)PMMA from saline vehicle, the PMMA stimulus generalized to PMMA-AT (ED_{50} = 0.29 mg/kg)

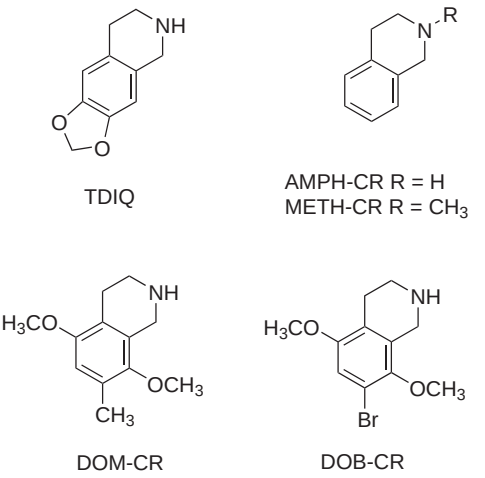
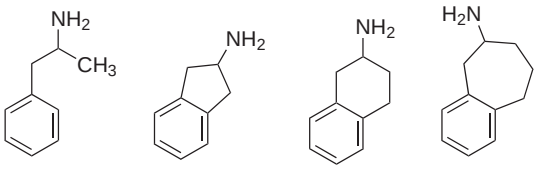


Figure 4-13. Chemical structures of TDIQ, and conformationally constrained forms of amphetamine (AMPH-CR), methamphetamine (METH-CR), and the hallucinogens DOM (DOM-CR) and DOB (DOB-CR).

with this agent being essentially equipotent with PMMA (ED_{50} = 0.41 mg/kg) [14]. In contrast, the PMMA stimulus failed to fully substitute for either TIQ analog. The results suggested that the stimulus effects of PMMA are associated more with an aminotetralin-like conformation (as shown by Rotamer 1) than with a tetrahydroisoquinoline-type of conformation of the molecule [14].

However, it should not be implied from the above results that "inactive" conformationally constrained rotamers are necessarily pharmacologically inert. For example, rats have been trained to discriminate 5.0 mg/kg of TDIQ (see Figure 4-13 for structure, and Figure 3-6 for learning data) from saline vehicle. Although the exact mechanism of action of TDIQ has yet to be determined, it would appear that it involves stimulation or partial agonism of α_2-adrenoceptors. The TDIQ stimulus generalized to TIQ-1 (ED_{50} = 1.6 mg/kg) but not to PMMA (maximum TDIQ-appropriate responding = 13%) (see Figure 4-13 for structure) [15]. These results are just the opposite of what was found when the agents were administered to PMMA-trained animals. Likewise, although a (+)amphetamine stimulus failed to generalize to the conformationally constrained (tetrahydroisoquinoline-like) analogs of amphetamine and methamphetamine, AMPH-CR and METH-CR, respectively (see Figure 4-13 for structures), the TDIQ stimulus generalized to AMPH-CR (ED_{50} = 1.5 mg/kg), but not to METH-CR (which produced a maximum of 6% TDIQ-appropriate responding) [15]. As an aside, it might be noted that introduction of a methyl group, comparing METH-CR with AMPH-CR, completely altered the stimulus nature of the agent. Conformationally constrained analogs of the hallucinogens DOM and DOB (i.e., DOM-CR and DOB-CR) were not recognized by rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle, but substituted in rats trained to discriminate TDIQ from vehicle (ED_{50} = 4.2 and 3.4 mg/kg, respectively) [15]. Interestingly, the TDIQ stimulus did not generalize to the *N*-methyl analog of



AMPH AMPH-IN AMPH-AT AMPH-BC

Figure 4-14. The chemical structure of amphetamine and three conformationally constrained analogs.

DOM-CR [15]. Taken together with the findings obtained for METH-CR, it would appear that *N*-methylation is not tolerated with regard to producing TDIQ-like discriminative stimulus effects [15]. But, more importantly, and to reiterate what was stated above, it should not be assumed that "inactive" conformationally constrained rotamers are necessarily pharmacologically inactive; results depend on the similarity in the stimulus properties of the training drug and test agent.

Another comparison of conformationally constrained analogs (i.e., "locked" *rotameric* forms of a molecule) examined the indane analog (AMPH-IN), the aminotetralin analog (AMPH-AT) and the benzocycloheptane analog (AMPH-BC) of amphetamine (Figure 4-14) [4]. Whereas no additional carbon atoms were introduced to amphetamine to form AMPH-IN, the distance from the amine to the aromatic centroid is decreased somewhat relative to the corresponding distance in amphetamine. On this basis, it might be expected that AMPH-IN will be less potent (or inactive) relative to amphetamine. On the other extreme, AMPH-BC represents a different molecular geometry than that found with AMPH-IN (i.e., a more out-of-plane orientation of the amine relative to the aromatic ring), but, at the same time, the structure introduces two additional carbon atoms that may or may not be tolerated. A (+)amphetamine stimulus generalized to AMPH-IN (ED_{50} = 2.12 mg/kg; 12.5 μmoles/kg) and AMPH-AT (ED_{50} = 1.20 mg/kg; 6.6 μmoles/kg), but neither agent was as potent as racemic amphetamine (ED_{50} = 0.62 mg/kg; 2.6 μmoles/kg). Perhaps a more out-of-plane, perhaps *gauche*, side chain conformation is preferred. This is more closely mimicked by AMPH-BC than AMPH-AT. However, AMPH-BC produced saline-appropriate responding at doses of up to 20 mg/kg [4]. Because AMPH-AT was less potent than AMPH, there are two possible conclusions. First, a more out-of-plane conformation is preferred (as closely mimicked by AMPH-BC), but the introduction of the two carbon atoms (i.e., an "ethylene bridge" as found in AMPH-BC) is simply not tolerated. Second, introduction of even a single methylene group *ortho* to the side chain results in reduced potency. Actually, the latter concept is consistent with the reduced potency of oTAP (Figure 4-1) (ED_{50} = 4.1 mg/kg; 22 μmoles/kg) relative to amphetamine. Nevertheless, the issue remains unresolved. But, for the sake of argument, let's assume that AMPH-AT was known prior to the discovery of AMPH. In this case, ring-opening of AMPH-AT to AMPH would have resulted in increased potency. This would represent an example of where release of conformational constraint was beneficial.

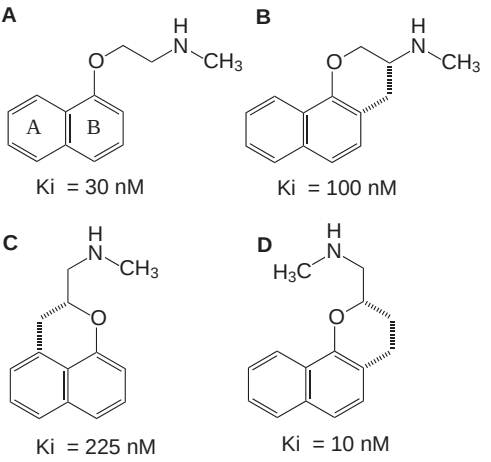


Figure 4-15. Conformationally constrained analogs of the flexible 5-HT_{1D} serotonin receptor ligand shown as "A." The hatched lines indicate how the structure of "A" was conformationally constrained, and K_i values for 5-HT_{1D} receptor binding are provided. Each of the structures (i.e., B–D) is chiral; that is, each consists of a pair of optical isomers, individual optical isomers were not prepared and examined; hence, affinity could be even further improved over that shown [16].

A general premise is that a conformationally constrained analog of a given conformationally flexible agent *might* be equipotent or more potent than the flexible agent *if* it mimics the *biologically preferred* conformation, but will be less effective, or ineffective, if it does not. However, results should be interpreted cautiously and conservatively; lack of effect of a conformationally constrained analog (given caveats already discussed) does not necessarily mean that the conformation (represented by a conformationally constrained structure) is not the preferred conformation.

An interesting example is provided by the conformationally flexible 5-HT_{1D} receptor ligand shown as the structure in Figure 4-15A. What is the preferred conformation for binding? Several conformationally constrained analogs were considered (i.e., as shown in Figure 4-15B–D). It can be appreciated that several conformationally constrained analogs are possible. Each was synthesized and evaluated with regard to its ability to bind to human 5-HT_{1D} serotonin receptors. It is apparent that the structure shown as Figure 4-15D possesses the highest affinity for these receptors and, as a consequence, likely represents the biologically preferred conformer of the structure shown as Figure 4-15A. The point here is that had only two possible rotamers of a given agent been considered and only one of the two examined and found to be of reduced activity (e.g., potency, affinity), it should *not* be assumed that the other rotamer will be the "active" rotamer unless the appropriate compound(s) is prepared and examined. In the example just cited, if only two conformationally constrained compounds had been initially considered (e.g., B and C in Figure 4-15) and had only the structure shown as Figure 4-15B been examined, it might have been erroneously assumed that

the structure shown as Figure 4-15C represents the "active" conformer. It was only after subsequent evaluation of the latter compound that allowed the conclusion to be reached that neither might represent the "active" conformation of the initial agent, and that other rotameric analogs required examination. It might also be noted that whereas two of the conformationally constrained analogs introduced only a single new methylene bridge (4-15B and C), the higher-affinity compound possesses an ethylene bridge.

2. Configurational Isomers

Configurational isomers (also referred to as "*geometric isomers*") are stereoisomers that are separated by high energy barriers and that are not interconvertable under ordinary conditions (i.e., bond rupture would be required). Such isomers are commonly found with agents possessing a double bond or a cyclic structure. Configurational isomers need not (but can, infrequently) possess similar pharmacological actions.

The chemical names of configurational isomers are typically preceded by the terms *Z* (or *zusammen* = same) or *E* (*entgegen* = opposite) [1]. In simple cases, terms such as *cis* and *trans* might be used. Figure 4-16A shows the two possible configurational isomers for 2-butene: CH_3-CH=CH-CH_3. The isomer where the two terminal methyl groups are on the same face of the molecule is termed the *cis* or *Z* isomer, whereas the

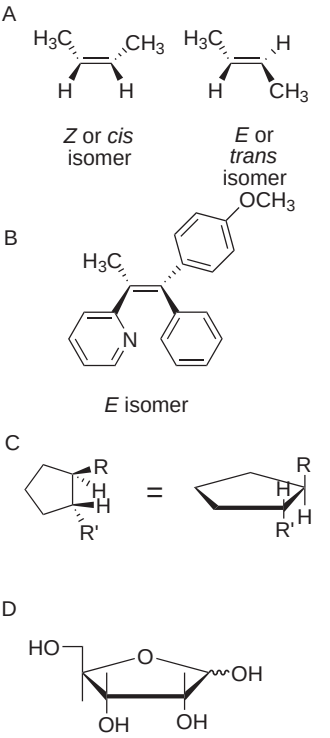
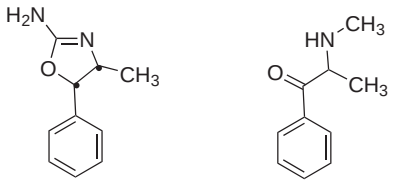


Figure 4-16. Examples of some configurational isomers.



4-Methylaminorex Methcathinone

Figure 4-17. The chemical structure of the designer drugs 4-methylaminorex and methcathinone (MCAT). With 4-methylaminorex, the methyl and phenyl groups can either be on the opposite (i.e., trans or E isomer) or the same (i.e., cis or Z isomer) face of the five-membered fing.

opposite isomer is the *trans* or *E* isomer. In more complicated cases, *E*/*Z* nomenclature is employed and substituent selection rules [1] are required to determine the priority of pendant substituents in order to correctly name an isomer (e.g., Figure 4-16B). Cycloalkanes can also be designated as *cis* and *trans* isomers. Shown by two equivalent representations in Figure 4-16C is a substituted cyclopentane analog where the R and R' substituents are *trans* to one another; whereas the rendering on the left is common, that on the right can be found in the older literature and is not commonly employed today (except in special cases as, for example, with sugars or nucleosides; see Figure 4-16D for the structure of ribose).

The designer drug 4-methylaminorex ("U4Euh"; 4-MAX), a central stimulant, is a cyclic structure that consists of *cis* and *trans* isomers (Figure 4-17). The stimulus-generalization properties of the *cis*, *trans*, and the isomeric mixture of 4-methylaminorex were examined in rats trained to discriminate 1.0 mg/kg of (+)amphetamine from saline vehicle. The (+)amphetamine stimulus generalized to each suggesting that 4-methylaminorex is an amphetamine-like stimulant [17]. Actually, the chemical composition is more complicated than described here and more will be said about 4-methylaminorex later (see section 4. below).

Rats trained to discriminate 8.0 mg/kg of the central stimulant cocaine from saline vehicle were used to further examine the stimulus properties of *cis*(±)4-methylaminorex and the structurally related stimulant (±)methcathinone (MCAT, CAT or *N*-methylcathinone; structure shown in Figure 4-17). The stimulus properties of these controlled substance analogs were compared with those of their parent compounds aminorex and (±)cathinone, respectively (see Figure 4-18 for the chemical structure of the latter). All agents resulted in cocaine-stimulus generalization with the following rank order of potency: aminorex (ED_{50} value = 0.8 mg/kg) > methcathinone (1.9 mg/kg) > cathinone (3.7 mg/kg) > *cis*(±)4-methylaminorex (5.2 mg/kg) > cocaine (7.6 mg/kg) [18].

Methcathinone (Figure 4-17) is a CNS stimulant that was a significant drug of abuse in the former Soviet Union. It appeared on the clandestine market in the United States and is now classified as a Schedule I substance. *S*(−)Methcathinone [*S*(−)MCAT, 0.50 mg/kg] was employed as a training drug in a two-lever drug discrimination task in rats. In tests of stimulus generalization, the *S*(−)MCAT (ED_{50} = 0.11 mg/kg)

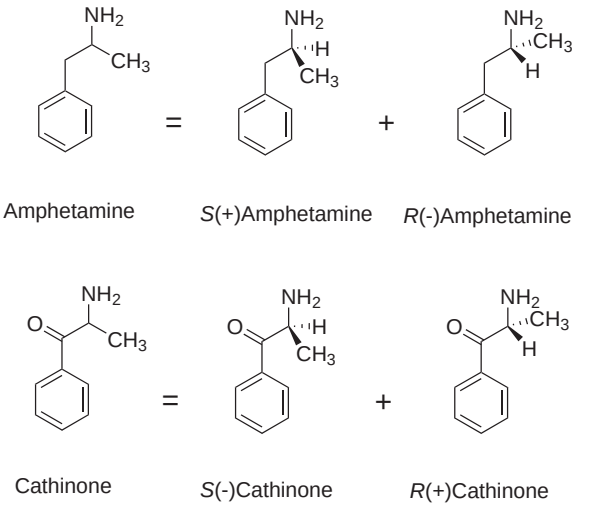


Figure 4-18. Structures of racemic, S(+)- and R(–)amphetamine, and racemic, S(–)- and R(+) cathinone.

stimulus generalized to *S*(+)methamphetamine (ED_{50} = 0.17 mg/kg), *S*(–)cathinone (ED_{50} = 0.19 mg/kg), *S*(+)amphetamine (ED_{50} = 0.23 mg/kg), aminorex (ED_{50} = 0.27 mg/kg), (±)MCAT (ED_{50} = 0.25 mg/kg), (±)cathinone (ED_{50} = 0.41 mg/kg), *R*(+)MCAT (ED_{50} = 0.43 mg/kg), *cis*-4-methylaminorex (ED_{50} = 0.49 mg/kg), methylphenidate (ED_{50} = 0.83 mg/kg), and cocaine (ED_{50} = 1.47 mg/kg) [19]. Haloperidol (AD_{50} = 0.18 mg/kg), a dopamine receptor antagonist, potently antagonized the *S*(–) MCAT stimulus indicating that MCAT and agents to which the MCAT-stimulus generalized may exert their stimulus effects, at least in part, through a dopaminergic mechanism [19].

3. Optical Isomers

Optical isomers are stereoisomers which, from a physicochemical perspective, differ with respect to the direction in which they rotate the plane of polarized light when examined in solution. When most non-chemists think of the term "*isomer*," they usually envision optical isomers. It now should be readily apparent that there are several types of stereoisomers, and that optical isomers constitute only one of several classes of isomers. Certain compounds possess a *chiral* center (e.g., a carbon atom with four different substituents)—though it should be realized there are other means of achieving chirality. A substance with a chiral center, for reasons of synthetic simplicity, is often prepared as its *racemate*. A racemate or "*racemic mixture*" is a *combination of equal amounts of the two optical isomers*. It should not be assumed that half the pharmacological action of a racemate rests with one isomer and half with the other; this is incorrect. In extreme cases, the action rests with one isomer whereas the other is inactive (see subsequent discussion of *stereoselectivity versus stereospecificity*). Optical isomers

can often be separated (i.e., *resolved*) by one of several methods, or prepared individually by *stereoselective synthesis*. Due to the added cost, labor, and/or synthetic complexity associated with resolution or stereoselective synthesis, racemates are commonly prepared and used for initial pharmacological evaluations. Very often, it is only after a racemate shows the desired pharmacological action that its individual optical isomers are prepared and evaluated. And, even then, this might not happen on a routine basis.

Substances that are chiral are mirror images of each other. They are said to possess handedness [1]. Many therapeutic agents are chiral and one of the *enantiomers* or optical isomers of the isomeric pair, found in a racemic mixture, may (predominantly) exhibit the desired pharmacologic property. Indeed, in some cases, one enantiomer may be far less potent, be inactive, or exert a different type of biological activity than the opposite enantiomer. Unfortunately, individual optical isomers of a racemate might not be examined. In contrast, investigators examining optical isomers sometimes fail to examine the racemate at the expense of only examining individual isomers. Valuable information might be missed. For example, one isomer might potentially antagonize or synergize the effects of the other isomer; this would be seen most clearly by comparing the actions of both isomers with those of the racemate. That is, if the racemate is more potent than either isomer, this would suggest that one isomer is capable of potentiating the effect of the other. Conversely, if the racemate is substantially less potent than its more potent isomer, the opposite (i.e., "inactive") isomer might be acting to antagonize the actions of the "active" isomer.

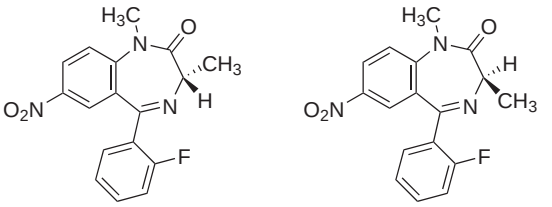
The naming of optical isomers can be confusing. One commonly employed method differentiates isomers of a given agent according to the direction of rotation of polarized light. Hence they are termed (+) or "*d*" for *dextrorotatory* and (–) or "*l*" for *levorotatory*. This is determined by examining solutions of the isomers using an instrument called a *polarimeter*. The terms "*d*" and "*l*" have fallen into disuse and have been largely replaced by "(+)" and "(–)," respectively. Certain older agents, by virtue of tradition, habit, or long term usage, are still occasionally referred to as, for example, "*d*"; an example is "*d*-amphetamine." Nevertheless, "*d*" or "(+)," and "*l*" or "(–)," mean the same thing. The name of a racemic mixture of a given compound may be prefixed by "(±)" or "(+/–)"; however, it is implied or understood that reference is being made to the racemate when a descriptor is not used. That is, the "(±)" term is normally used only to differentiate the racemate (i.e., racemic mixture) from an isomer when the racemate and isomers are included in the same study.

Shown in Figure 4-18 are the structures of racemic, *S*(+)- and *R*(–)amphetamine. Note, the racemate is not provided with a descriptor here, but could have been termed (±)amphetamine. The "*S*" and "*R*" nomenclature will be described later. If a descriptor is not used (as in Figure 4-18 for amphetamine and cathinone), the racemate is implied by default. The individual optical isomers of amphetamine can be obtained by resolution of the racemate or by stereoselective synthesis. Clinically, (+)amphetamine is known as dextroamphetamine, and (–)amphetamine, although not used clinically in the United States, is known as levamphetamine. These isomers are also referred to as *d*-amphetamine and *l*-amphetamine, respectively. These isomers have the same empirical formula ($C_9H_{13}N$ for the free base) and similar physicochemical properties except for the direction in which solutions of the isomers rotate in the plane of polarized light as

measured using a polarimeter. But, the pharmacological properties of isomers can dramatically differ.

"Stereoselectivity" versus "stereospecificity": In general, if only one of the two isomers of a racemate is active in producing a given effect, its opposite enantiomer serves only to dilute the effect (unless the inactive isomer somehow potentiates or antagonizes the action of the active isomer). Hence, the active isomer should be, at most, only twice as potent as the racemate (i.e., the inactive isomer serves as diluent). On the other hand, both isomers might produce a similar effect but one isomer is more potent than the other. In this case, analysis of the actions of the racemate might be more complicated. If both optical isomers of a given agent produce a common effect but one isomer is more potent than the other, this is termed "*stereoselectivity*." In contrast, if one isomer produces a given effect but its opposite enantiomer does not (i.e., it fails to produce the effect), this is termed "*stereospecificity*." Amphetamine is a central stimulant. Are the actions of its individual optical isomers stereoselective or stereospecific? The stimulant actions of amphetamine are usually attributed primarily to its (+)-isomer. However, with regard to cardiovascular actions, the two isomers have been found equipotent. Using animals trained to discriminate (+)amphetamine from saline vehicle, it should be possible to determine which of the two amphetamine optical isomers is responsible for its stimulus actions. A number of investigators have examined the stimulus effects of amphetamine and its isomers in animals trained to discriminate (±)- or *S*(+)amphetamine from vehicle [reviewed: 4]. In general, using (+)amphetamine-trained animals, (+)amphetamine is twice as potent as its racemate, and at least three times more potent than (–)amphetamine [4]. That is, the stimulus effects of amphetamine are stereoselective with both isomers producing common stimulus effects, but with (+)amphetamine being more potent than (–)amphetamine. In one drug discrimination study it was determined that the ED_{50} values for stimulus generalization were (+)AMPH (ED_{50} = 0.42 mg/kg) > (±)AMPH (ED_{50} = 0.62 mg/kg) > (–)AMPH (ED_{50} = 1.23 mg/kg) [4]. Hence, the stimulus properties of amphetamine isomers are consistent with what has been reported earlier regarding their central stimulant potencies *versus* their cardiovascular effects.

How does stereospecific generalization (substitution) differ from stereoselective generalization? 3-Methylflunitrazepam (Figure 4-19) is one of the few benzodiazepine anxiolytic agents that possesses a chiral center. In rats trained to discriminate 3.0 mg/kg of diazepam from saline vehicle, the diazepam stimulus generalized to the



S(+)-3-Methylflunitrazepam *R*(-)-3-Methylflunitrazepam

Figure 4-19. Optical isomers of 3-methylflunitrazepam.

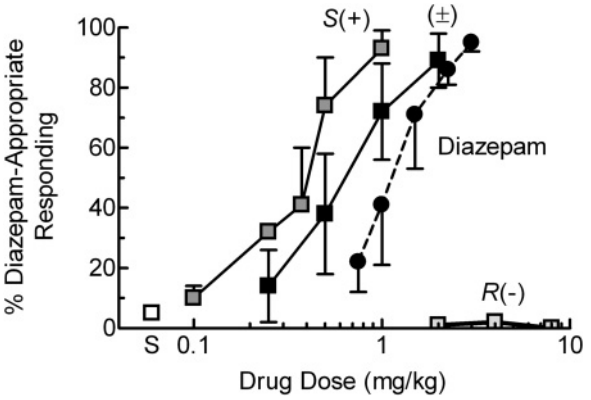


Figure 4-20. Effect of (±)3-methylflunitrazepam and its optical isomers S(+)- and R(–)-3-methylflunitrazepam in rats trained to discriminate 3.0 mg/kg of diazepam from saline vehicle. S = effect of saline.

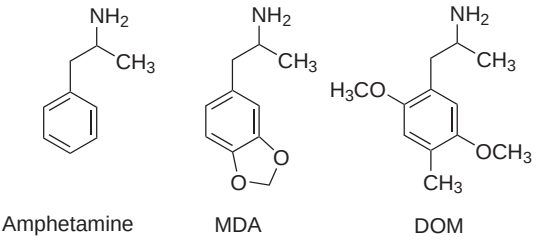


Figure 4-21. A comparison of the chemical structures of the central stimulant amphetamine, MDA, and the hallucinogen DOM.

S(+)-isomer, but not to the *R*(−)-isomer of 3-methylflunitrazepam (Figure 4-20). The *S*(+)-isomer (ED_{50} = 0.34 mg/kg) was twice as potent as the racemate (ED_{50} = 0.65 mg/kg), whereas the *R*(−)-isomer elicited saline-appropriate responding at doses of up to nearly 25 times that of the *S*(+)-isomer [20]. This is an example of where substitution occurred in a stereospecific manner and where the "inactive" isomer served as diluent when the racemate was examined.

Stereoselective *versus* stereospecific substitution can also depend on the nature of the training drug. For example, with rats trained to discriminate 3.0 mg/kg of 5-OMe DMT (see Figure 4-6 for chemical structure) from saline vehicle, substitution occurred upon administration of *R*(−)DOM (ED_{50} = 0.38 mg/kg) whereas administration of *S*(+)DOM produced saline-appropriate responding followed, at higher doses, by disruption of the animals' behavior [5]. That is, substitution was stereospecific. In contrast, using rats trained to discriminate 1.0 mg/kg of DOM (see Figure 4-21 for chemical structure), to which 5-OMe DMT substitution occurred, substitution occurred to both optical isomers of DOM (i.e., ED_{50} = 0.21 and 1.70 mg/kg for *R*(−)- and *S*(+)DOM,

respectively). Here, substitution was stereoselective. This suggests that, at the training doses employed, there are similarities, but also differences, between the stimulus effects produced by these two training drugs. This concept is further supported by the finding that, when administered to 5-OMe DMT-trained rats, both optical isomers of DOET (i.e., the 4-ethyl homolog of DOM) produced vehicle appropriate responding, whereas substitution in DOM-trained animals was stereoselective (ED_{50} = 0.09 and 0.85 mg/kg for *R*(−)- and *S*(+)DOET, respectively [5]. Opposite results were obtained with 6-OMe DMT (see Figure 4-6 for chemical structure). Whereas a 5-OMe DMT stimulus generalized to 6-OMe DMT (*vide supra*), the DOM stimulus did not [4]. Obviously, as already mentioned, the identity of the training drug plays a substantial role in drug discrimination studies, and different results can be obtained when two "similar" agents (i.e., agents that seemingly produce similar stimulus effects as determined by mutual substitution) are used as training drugs.

Another interesting example of stereospecific generalization derives from investigations with the abused substance MDA [1-(3,4-methylenedioxyphenyl)-2-aminopropane] (Figure 4-21). MDA, whose initial popularity arose during the 1960s, is claimed in anecdotal reports to produce effects in humans akin to ingestion of a combination of a central stimulant and a hallucinogenic agent. Using rats trained to discriminate the central stimulant (+)amphetamine from vehicle, the amphetamine stimulus generalized to (±)- and *S*(+)MDA in a dose-related manner [21, 22]. Substitution did not occur to the *R*(−)-isomer. Figure 4-22 shows the highest percent drug-appropriate responding obtained. In contrast, using rats trained to discriminate 1.0 mg/kg of the hallucinogen DOM from vehicle, the DOM stimulus generalized to (±)- and *R*(−)MDA in a dose-related manner [23]. DOM-stimulus generalization did not occur upon administration of doses of the *S*(+)isomer [23]. Consequently, generalization was stereospecific in both groups of animals. But, one isomer behaved in a stereospecific fashion in one group whereas it was its opposite enantiomer that was stereospecific in the other group of animals. *Stereospecificity, then, is not solely a property of the drug, but it is related both to the drug and the pharmacologic action being examined*. Further evidence supporting this conclusion is that with animals trained to discriminate (±)MDA from saline vehicle, the MDA stimulus generalized to (±)-, *R*(−)- and *S*(+)MDA [24]. Again emphasizing that different optical isomers can behave differently depending upon the specific training drug being employed.

Perhaps the strongest evidence for stereospecificity of effect was the ability to train rats to discriminate between the optical isomers of MDA in a three-lever operant procedure [25]. That rats could be trained to discriminate *R*(−)MDA from *S*(+)MDA from vehicle indicated that their stimulus effects are not identical. Predictably, administration of (±)MDA resulted in the animals dividing their responses nearly equally between the *R*(−)MDA- and *S*(+)MDA-designated levers. Furthermore, when administered various agents in tests of stimulus generalization, the animals responded on the *R*(−)MDA-appropriate lever when administered hallucinogens such as DOM, mescaline, and LSD, and responded on the *S*(+)MDA-appropriate lever when administered stimulants such as (+)amphetamine or cocaine [25]. This was the first instance in which it was shown that animals can be trained to discriminate between the optical isomers of the same substance in a three-lever operant paradigm.

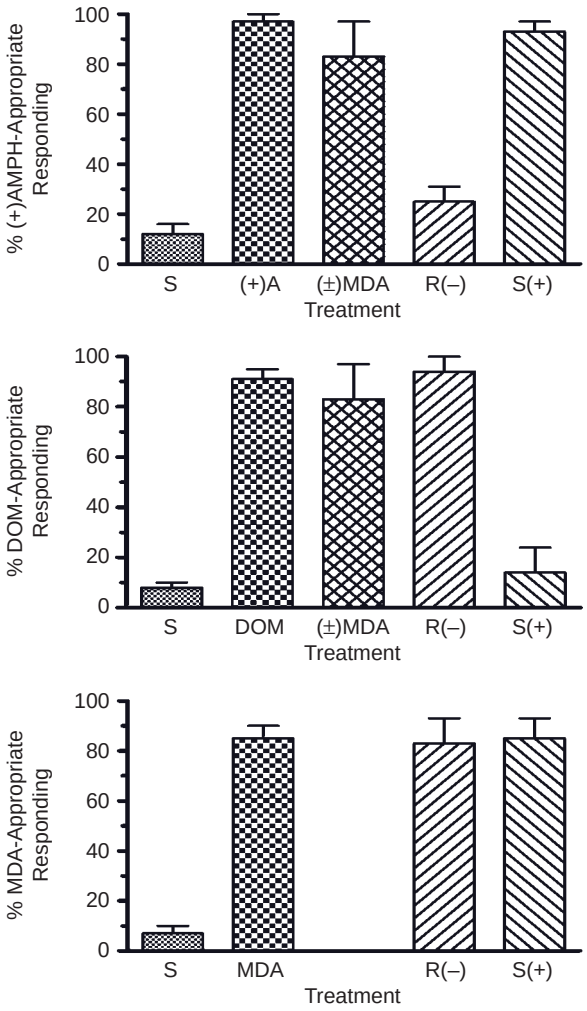


Figure 4-22. Effect of (±)-, R(–)- and S(+)MDA (labeled R(–) and S(+), respectively) in rats trained to discriminate either (+)amphetamine (1.0 mg/kg) (top), DOM (1.0 mg/kg) (center), or MDA (1.5 mg/kg) (bottom) from saline vehicle. Full dose-response curves were obtained for each agent but data are shown for the highest drug-appropriate responding that did not result in behavioral disruption in a majority of animals. See text for additional discussion.

It is commonly held that if an agent possesses a chiral center, and if the agent's actions are receptor-mediated, there should exist a difference in the action or receptor affinity of the agent's individual isomers. This is most often the case. For example, in the early days of receptor research employing radioligand binding techniques, it was thought that failure to observe stereoselectivity or stereospecificity of binding of an optically- active radioligand might be indicative of nonspecific binding. A final issue

that should be appreciated is the concept of "*critical*" and "*noncritical*" chiral centers [26, 27]. In some instances, the chiral center of a pharmacologically- active agent might not be situated such as to directly influence interactions with the receptor (or is situated near a region of the molecule not directly interacting with a specific receptor-associated amino acid residue—such as in a region of "bulk tolerance") and, hence, there might be no difference in the activity/affinity of the individual optical isomers. That is, for agents with a noncritical chiral center, both isomers might display identical activity/affinity. As a consequence, the individual isomers of optically active agents might display pharmacological stereoselectivity, stereospecificity, or no difference at all.

Absolute Configuration A second method for identifying optical isomers is by their absolute configuration. Early on, the absolute configuration of a substance was related to that of glyceraldehyde which served as a standard [1]. Glyceraldehyde is chiral (i.e., optically active), and its two isomers were arbitrarily labeled D and L. In this system, compounds are named by analogy to glyceraldehyde. The D and L labels are unrelated to the *d* and *l* or (+) and (–) system. That is, D ≠ *d*, and L ≠ *l*. Rather, the stereochemistry of a compound is related (usually by degradation) to that of the D or L isomer of glyceraldehyde—the dextrorotatory isomer of glyceraldehyde was later, coincidentally, shown to be the D isomer. This system is now rarely employed except with certain chemicals, such as amino acids and sugars.

The *R* and *S* absolute configuration system is now considered the most important nomenclature for denoting the identity of enantiomers [1]. The designations derive from the Latin *rectus* (*R*) and *sinister* (*S*), right and left, respectively, and refer to the spatial orientation of groups at a chiral center, and *not* to the direction of rotation of polarized light. Hence, the *R* or *S* and (+) or (–) systems are independent of one another, and it is possible for an isomer to be *S*(+), *S*(–), *R*(+) or *R*(–). This system names each chiral center in a molecule according to specific priority rules referred to as the *Cahn-Ingold-Prelog sequence* (or *chirality*) *rules* [28]. Whereas it is fairly trivial to determine whether a given isomer is (+) or (–) using a polarimeter, its optical rotation provides no information about chemical structure (i.e., absolute configuration). In contrast, determination of absolute configuration provides information about chemical structure in three-dimensional space, but additional instrumental or chemical means are required to determine this experimentally. As a consequence, it is more common to see the name of an isomer preceded by (+) or (–) than by *R* or *S*. The term "*RS*" refers to a racemic mixture and is equivalent to "(±)", but the use of the latter is more common than that of the former. The most accurate description of stereochemistry provides both its absolute configuration (*R* or *S* when possible) and the direction is which solutions of the agent rotate the plane of polarized light. Hence, (+)amphetamine, dextro-amphetamine, or *d*-amphetamine is *S*-amphetamine and is most appropriately referred to as *S*(+) amphetamine. Its opposite enantiomer is *R*(–)amphetamine. Different techniques are required to determine the absolute configuration of an isomer and the direction in which solutions of the isomer rotate the plane of polarized light.

Cathinone (Figure 4-18) is an interesting example that can serve to illustrate these concepts and highlight how confusion might occur. Cathinone, actually its levorotatory or (–)isomer, (–)cathinone, is a naturally occurring constituent of the shrub *Catha edulis*

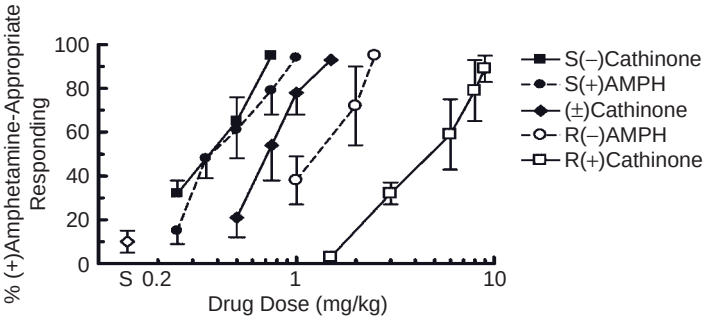


Figure 4-23. Results of stimulus generalization studies with cathinone in rats trained to discriminate 1.0 mg/kg of (+)amphetamine from vehicle. S = effect of saline administration.

(khat, qat) indigenous to Eastern Africa and the Arabian peninsula. The shrub is known for its central stimulant properties when chewed or prepared as a tea, and (–)cathinone was isolated and identified in the late 1970s. Due to its stimulant actions and structural similarity to amphetamine, it was termed a "naturally occurring amphetamine analog." However, the argument was raised by some investigators that this was unlikely to be the case because (–)cathinone was more potent than (+)cathinone whereas (+)amphetamine is more potent than (–)amphetamine as a central stimulant. For example, in drug discrimination studies (Figure 4-23), (–)cathinone (ED_{50} = 0.34 mg/kg in rats trained to discriminate 1.0 mg/kg of (+)amphetamine from saline vehicle) was more potent than its racemate (ED_{50} = 0.72 mg/kg), which was more potent than (+)cathinone (ED_{50} = 4.41 mg/kg) [29], whereas (+)amphetamine is more potent than (–)amphetamine. But, inspection of the actual structures of the cathinone isomers shows that (–)cathinone and (+)amphetamine both possess the same absolute configuration: *S* (Figure 4-18). *It is the three-dimensional structure of a molecule, not its optical rotation, that is most important for potency comparisons between agents.*

As further support for the concept that *S*(–)cathinone might be a naturally- occurring amphetamine-like agent, a structural modification known to retain or enhance the stimulant properties of amphetamine was introduced to the structure of cathinone; specifically, *N*-monomethylcathinone (see Figure 4-17 for chemical structure) was prepared and its actions compared with *N*-monomethylamphetamine (methamphetamine). By analogy to the latter, the former was termed methcathinone [30]. Methcathinone was found more potent than cathinone with regard to its ability to release dopamine, stimulate mouse locomotor activity, and substitute for a (+)amphetamine stimulus in rats [30]. Examined in mouse locomotor activity studies and stimulus generalization studies employing rats trained to discriminate (+)amphetamine, *S*(–)methcathinone was more potent than *R*(+)methcathinone [31]. Likewise, both methcathinone optical isomers substituted in cocaine-trained rats and, here too, *S*(–)methcathinone was more potent than *R*(+)methcathinone [31]. Subsequently, *S*(–)methcathinone was used as a training drug and stimulus generalization occurred to a number of stimulants with the following rank order of potency: *S*(–)methcathinone > *S*(+)methamphetamine > *S*(–)cathinone > *S*(+)amphetamine > *R*(+)methcathinone > cocaine [32]. All of these studies

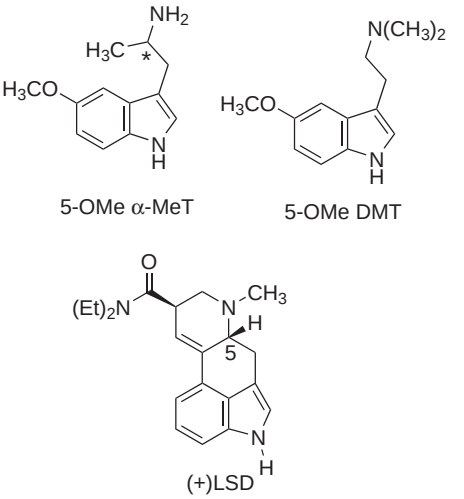


Figure 4-24. Chemical structures of 5-methoxy-α-methyltryptamine (5-OMe α-MeT; asterisk denotes chiral center), 5-methoxy-N,N-dimethyltryptamine (5-OMe DMT), and (+)lysergic acid diethylamide [(+)LSD].

indicated that cathinone and methcathinone are amphetamine-like stimulants and produce amphetamine-like stimulus effects, and that their *S*-isomers are the more potent in each instance (regardless of the direction in which solutions of the agents rotate the plane of polarized light!).

Anecdotal evidence suggested that 5-methoxy-α-methyltryptamine (5-OMe α-MeT) (Figure 4-24) is a hallucinogenic agent with actions similar to those of (+)LSD and 5-methoxy DMT (5-OMe DMT). 5-OMe α-MeT possesses a chiral center (see Figure 4-24) and exists as two optical isomers. It was hypothesized that the more potent isomer of 5-OMe α-MeT should possess the same stereochemistry about the 5-position of (+)LSD in three-dimensional space (i.e., with the hydrogen atom in front of the plane of the molecule, as seen with LSD). The absolute configuration of the 5-OMe α-MeT isomer with this stereochemistry is the *S*(+)isomer. Both isomers and the racemate of 5-OMe α-MeT were examined in rats trained to discriminate DOM from saline vehicle [33]; data for (+)LSD and 5-OMe DMT are provided here for purpose of comparison in Figure 4-25.

By examining the racemate of 5-OMe α-MeT in addition to its two individual optical isomers, the potency relationship is clear. For comparison, the ED_{50} values for (+)LSD and 5-OMe DMT were 0.05 and 1.22 mg/kg, respectively. That is, *S*(+)5-OMe α-MeT (ED_{50} = 0.21 mg/kg) was found to be about twice as potent as the racemate (ED_{50} = 0.52 mg/kg) and nearly 5 times more potent than its *R*(−)enantiomer (ED_{50} = 0.92 mg/kg) [33]. It might be noted that the isomers of 5-OMe α-MeT have since been evaluated in human subjects and *S*(+)5-OMe α-MeT was found to be "*clearly three to four times more potent than the R-isomer*" [34]. Likewise, the DOM stimulus generalized to the *des*-methoxy analog of 5-OMe α-MeT (i.e., racemic α-MeT;

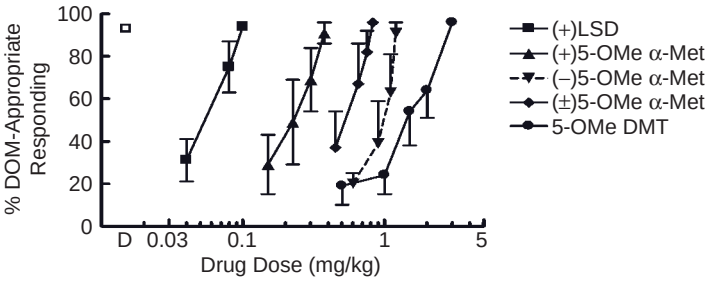


Figure 4-25. Results of stimulus generalization studies with (±)-, (+)-, and (–)5-OMe α-MeT, 5-OMe DMT, and (+)LSD in rats trained to discriminate 1.0 mg/kg of DOM (D) from saline vehicle. Saline produced <20% DOM-appropriate responding (data not shown).

ED_{50} = 3.13 mg/kg) and its *S*(+)-isomer (ED_{50} = 1.64 mg/kg), whereas administration of the *R*(–)-isomer of α-MeT resulted only in partial (61%) generalization (at a dose of 3.25 mg/kg); administration of a higher dose (3.5 mg/kg) of this isomer resulted in behavioral disruption [35]. The results were in accord because the *absolute configuration* about the chiral center (*not* the *optical rotation*) of the α-methyltryptamines is the same as that about the corresponding chiral center in (+)LSD. This is exactly what would have been expected. That is, once again, absolute configuration, not optical rotation, should be considered when comparing various agents. Absolute configuration is absolute with respect to three-dimensional structure, optical rotation is a relative term that is unrelated to absolute configuration. Many other examples can be provided, but hopefully, these will suffice.

4. Diastereomers

Up to this point, compounds possessing only a single chiral center have been discussed. Molecules can certainly possess more than one chiral center. And, in theory, the number of possible isomers is 2^n where n = the number of chiral centers (this is not necessarily true where the chiral center is at a "*bridgehead atom*") [1]. Thus, with one chiral center, two optical isomers are generally possible, with two chiral centers four isomers are possible, with three chiral centers eight isomers are possible, and so on. It might be noted that as the number of chiral centers increases, it becomes increasingly difficult to assign absolute configuration. Oft times, agents with multiple chiral centers are simply designated as (+) or (–) until absolute configuration can be determined. For example, (+)LSD is equivalent to 5*R*,8*R*-LSD. If an agent possesses four chiral centers, 16 optical isomers are possible and it would take a considerable effort to determine the absolute configuration at each chiral center; hence, here, an agent might simply be referred to as (+) or (–). Some chemists relish the idea of identifying the absolute stereochemistry of agents with multiple chiral centers (typically natural products), and those involved in drug design are indebted to them for their efforts. This can be a very laborious task to which many a doctoral dissertation has been devoted.

With respect to absolute configuration, consider, for example, the relatively simple case of phenylpropanolamines related in structure to amphetamine or, more accurately, methamphetamine. The general *N*-methylphenylpropanolamine structure, as drawn in Figure 4-26 (upper left of the figure) has two chiral centers and, thus, consists of four optical isomers. Two of the isomers are termed ephedrine: (–)- and (+)ephedrine, and two are termed pseudoephedrine: (–)- and (+)pseudoephedrine. See Table 4-1 for assignment of absolute configuration.

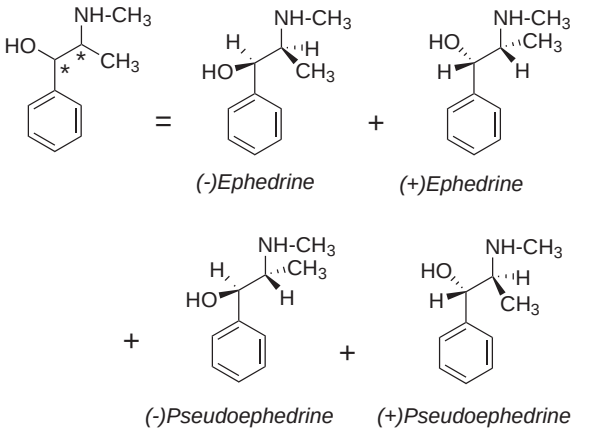


Figure 4-26. Chemical structures of four isomeric phenylpropanolamines. The general phenylpropanolamine structure (upper left) possesses two chiral centers as indicated by asterisks; hence 2^n, or four, optical isomers are possible. These isomers are termed (–)- and (+)ephedrine, and (–)- and (+)pseudoephedrine.

TABLE 4-1. Results of stimulus generalization studies with phenylpropanolamine and N-methylphenylpropanolamine isomers using rats trained to discriminate either (–) ephedrine (4.0 mg/kg) or (+)amphetamine (1.0 mg/kg) from saline vehicle

Agent	Absolute Stereochemistry	(–)Ephedrine-trained rats (ED_{50}; mg/kg)[a]	(+)AMPH-trained rats (ED_{50}; mg/kg)[b]
(–)Ephedrine	1*R*,2*S*	0.9	4.5
(+)Ephedrine	1*S*,2*R*	2.6	—
(–)Pseudoephedrine	1*R*,2*R*	—	—
(+)Pseudoephedrine	1*S*,2*S*	6.6	—
(–)Norephedrine	1*R*,2*S*	1.9	—
(+)Norephedrine	1*S*,2*R*	5.8	—
(–)Norpseudoephedrine	1*R*,2*R*	—	—
(+)Norpseudoephedrine	1*S*,2*S*	4.8	8.0
(+)Amphetamine	*S*	0.4	0.4

[a]Reference 37.
[b]Reference 38.

Diastereomers (diastereoisomers) are stereoisomers of identical constitution that differ in three-dimensional structure and that do not bear a mirror-image relationship to one another. For example, (–)- and (+)ephedrine (Figure 4-26) are optical isomers of identical constitution (i.e., of identical empirical formula = $C_{10}H_{16}NO$); and are mirror images of each other. On the other hand, ephedrine isomers and pseudoephedrine isomers possess a diastereomeric relationship (i.e., they are of identical composition with empirical formulae = $C_{10}H_{16}NO$), but are not mirror images of one another. The "generalized" *N*-methylphenylpropanolamine structure shown in Figure 4-26 (upper left) is actually a *diastereomeric mixture* of isomers. Because evaluation of *diastereomeric mixtures* can afford very misleading results (i.e., pharmacological results could reflect a combination effect of all four isomers, the actions of one isomer might be diluted by the other three, or the actions of one isomer might actually be synergized or antagonized by one or more of the other isomers present in the mixture), studies with diastereomeric mixtures are, as a rule, not conducted. This becomes even more complicated when there are more than two chiral centers present in a molecule. And, typically, such studies are not publishable because it would be similar to simultaneously examining the actions of a mixture of at least four agents at one time in the same assay. It is best not to evaluate diastereomeric mixtures. In extreme cases, an agent that possesses several chiral centers, and where configurational isomers are additionally possible, might actually be composed of 100 or more different isomers. If only one isomer is "active," its actions might be obscured or diluted by the other isomers present in the mixture. However, sometimes, diastereomeric mixtures are evaluated in pharmacological studies, including drug discrimination studies, to determine if the mixture has the desired effect. This is a "quick and dirty" study, is generally limited to agents with only two chiral centers, and then, if a desired effect is noted, individual isomers or diastereomers are examined to further clarify their actions [36]. Nevertheless, examination of diastereomeric mixtures is an infrequent practice.

All four *N*-methylphenylpropanolamine isomers have been examined in rats trained to discriminate (+)amphetamine from saline vehicle. The individual isomers have also been examined in rats trained to discriminate (–)ephedrine from saline vehicle. More will be said about these isomers in Chapter 6.

As mentioned earlier, stereospecificity and, for that matter, stereoselectivity, is/are *not* solely a property of a drug, but are related both to the drug and the specific pharmacologic action being examined. The use of different training drugs can afford dissimilar results. Four *N*-methylphenylpropanolamines are shown in Figure 4-26. There are four additional phenylpropanolamine isomers resulting from *N*-demethylation (e.g., norephedrine and its isomers; all possible isomers are show in Figure 6-8). That is, *N*-demethylation of ephedrine results in norephedrine, and *N*-demethylation of pseudoephedrine results in norpseudoephedrine. All eight agents (four *N*-methyl analogs and four *N-des*methyl analogs) were examined in tests of stimulus generalization in rats trained to discriminate either [1*R*,2*S*](–)ephedrine or *S*(+)amphetamine from saline vehicle [37, 38]. As shown in Table 4-1, the (–)ephedrine stimulus generalized to six of the eight agents. However, an *S*(+)amphetamine stimulus generalized only to two of the isomers: (–)ephedrine and (+)norpseudoephedrine [also known as (+)nor-Ψ-ephedrine or (+)cathine]. It might be noted that the latter two are the only isomers of

the eight examined that have been demonstrated to produce central stimulant activity in humans. In fact, of the latter two, (+)norpseudoephedrine (also known as cathine) has been isolated from aged samples of *Khat*. And, prior to the discovery of cathinone, cathine was thought responsible for the central stimulant actions of this plant product.

What this means is that caution should be exercised when declaring a drug's actions as being *stereoselective* or *stereospecific*. That is, the drug *and* the test system should both be defined. Stereoselectivity and stereospecificity are a property of the drug being evaluated *and* the test system in question.

What the results in Table 4-1 indicate is that some ephedrine/norephedrine isomers produced a (–)ephedrine-like effect, but that only two isomers, that is (–)ephedrine and (+)norpseudoephedrine [i.e., (+)cathine], produced a (+)amphetamine-like effect. Hence, the pharmacology and mechanism of action of these isomers must differ (see Chapter 6). The mechanism of action of these phenylpropanolamines has been examined [39].

The central stimulant and "*designer drug*" 4-methylaminorex ("U4EUh") was described above. It consists of *cis* and *trans* isomers (Figure 4-17). However, 4-methylaminorex also possesses two chiral centers and, as such, four optical isomers are possible. That is, the *trans* isomers can have their substituents located with the phenyl and methyl group being either in front of, or behind, the plane of the ring (i.e., one substituent on each side). With the *cis* isomers, these substituents are on the same side, both being in front or behind the plane of the ring. These are shown in Figure 4-27.

All four isomers of 4-methylaminorex were synthesized and examined in rats trained to discriminate 1.0 mg/kg of the central stimulant (+)amphetamine from saline vehicle [17]. The *S*(+)amphetamine stimulus generalized to all but one of the individual optical isomers following a 15-minute pre-session injection interval. The relative poten-

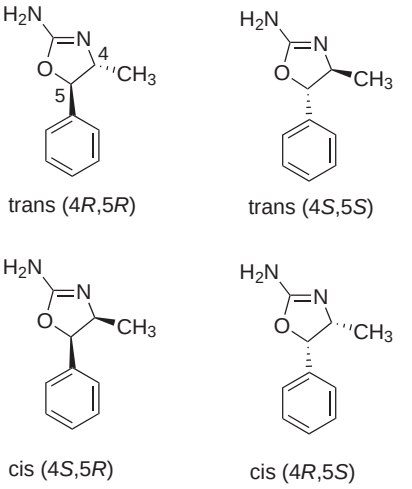


Figure 4-27. The four optical isomers of 4-methylaminorex.

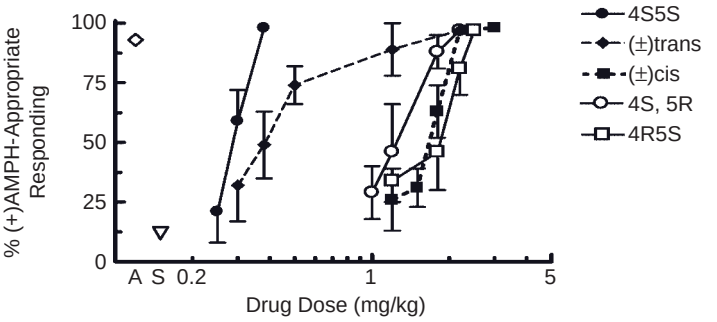


Figure 4-28. Results of stimulus generalization studies (PSII = 15 min) with 4-methylaminorex isomers in rats trained to discriminate 1.0 mg/kg of (+)amphetamine (A) from saline vehicle (S).

cies of the optical isomers (followed by ED_{50} values) were as follows: *trans*(4*S*,5*S*) (0.25 mg/kg) > *cis*(4*S*,5*R*) (1.2 mg/kg) = *cis*(4*R*,5*S*) (1.5 mg/kg) [17]. The *S*(+)amphetamine stimulus did not completely substitute for the *trans*(4*R*,5*R*) isomer unless a longer (i.e., 60-minute) pre-session injection interval was used suggesting that this isomer might have a longer duration of onset time than the other isomers of 4-methylaminorex. The results suggested that the *trans*(4*S*,5*S*) isomer (which is not classified as a Scheduled substance) is similar in potency to (+)amphetamine (ED_{50} = 0.4 mg/kg) and is more potent than either of the Scheduled *cis* isomers [17].

Another interesting feature, as revealed in Figure 4-28, is that all dose-response curves are parallel to one another except that for of the *trans* racemate. The *trans* racemate, being composed of the 4*S*,5*S* and 4*R*,5*R* isomers—the latter of which failed to substitute when administered alone following a 15-minute pre-session injection interval—seems to have altered the shape of the dose-response curve. Here is an instance where examination of a racemic mixture, in addition to the individual optical isomers, provided useful information.

5. Chiral Switch

Numerous other examples can be provided on the effect of stereochemistry (i.e., as it relates either to the training drug or test drugs) on the results of drug discrimination studies. And, additional examples will be subsequently provided. However, the examples in this chapter should serve as an illustration of the importance of stereochemistry in drug discrimination studies.

The chirality of drugs has emerged as an important consideration in drug design, discovery, and development. Historically, many therapeutic agents were manufactured and marketed as racemic mixtures. In some cases, pharmaceutical companies have investigated and invested in "*chiral switching*," preparation of a specific isomeric version of a drug that was originally marketed as a racemic mixture, with the goals of improving therapeutic efficacy and/or thwarting potential revenue losses caused by generic versions of the racemic mixture going "off patent" [e.g., 40]. For example,

chiral switching occurred with citalopram ((*RS*)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile; Celexa®), a selective serotonin reuptake inhibitor (SSRI) and antidepressant agent. The patent on citalopram expired in 2003 but in 2002 *S*(+)citalopram (*S*(+)-1-[3-(dimethylamino)propyl]-1-(4-fluorophenyl)-1,3-dihydroisobenzofuran-5-carbonitrile; escitalopram; Lexapro® or Cipralex®) was approved for the marketplace based on a more potent SSRI effect and reduced side effect profile than (±)citalopram. Other examples include *S*(+)ketamine, which purportedly exerts greater analgesic/anesthetic potency that is coupled with a reduced incidence of hallucinogenic activity and agitation as side effects than *R*(−) ketamine [e.g., 41, 42]. Also, the *R,R*-enantiomer of methylphenidate (*R,R*(+)methyl-2-phenyl-2-(2-piperidyl)acetate; dexmethylphenydate) is reported to be equally effective as *S,S*-methylphenydate at half the dose, with a more rapid onset of action, and improved side effect profile [43, 44]. However, not all "switches" have resulted in an enhancement of therapeutic action. For example, the development *R*(−)fluoxetine was terminated due to a significant increase in cardiac QT prolongation which might lead to increased risk of arrhythmias and sudden cardiac death [e.g., 45]. Lastly, the anti-appetite drug (±)fenfluramine was one of the first agents to undergo the chiral switch process with *S*(+)fenfluramine (dexfenfluramine) brought to the marketplace. Unfortunately, both the racemic mixture and dexfenfluramine were associated with valvular heart disease and were removed from the market [46]. Nevertheless, the shift toward the development of individual enantiomers and away from racemic mixtures is illustrated by the chirality characteristics of drugs that were approved worldwide from 1983 to 1986 compared to 1999 to 2002, respectively: 25% to 58% for single enantiomers, 32% to 8% for racemic mixtures, and 43% to 34% for achiral agents [47].

Without going into further detail, it should be recognized that stereochemistry plays a substantial role in drug action, and it can be readily appreciated how drug discrimination studies might be employed to evaluate the stimulus similarities (or differences), potencies, time of onset, and duration of action of individual isomers of an optically active substance using the racemate (or individual isomers) as training drug.

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5

DRUG DISCRIMINATION AND IN VIVO STRUCTURE–ACTIVITY RELATIONSHIPS

A. STRUCTURE–ACTIVITY CAVEATS

The drug discrimination paradigm has found wide application for the formulation of in vivo structure–activity relationships (SAR: i.e., the influence of chemical structure

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

on pharmacological activity—in this case, discriminative stimulus activity). Not only has it been possible to determine whether or not a test agent produces stimulus effects similar to those of a particular training dose of training drug, it also allows potency comparisons to be made by calculation and comparison of ED_{50} values. Similarly, AD_{50} doses can be calculated to compare potencies of antagonists. That is, *drug discrimination studies provide information that is both qualitative and quantitative*. It can be appreciated, then, that SAR studies, although perhaps of rather limited interest by themselves, constitute a very powerful tool when applied to, for example, drug design and examination of drug mechanisms.

An important caveat is that *SAR results might differ depending upon the training dose of the training drug*. Training drugs, depending upon their training dose, might produce different stimulus effects (see Chapters 1 and 3 for further discussion of this topic). The use of different pre-session injection intervals, optical isomers, related training drugs, and/or different animal species might also influence results. It also should be realized that the results of in vivo SAR might not be identical with results obtained from in vitro SAR conducted with the same agents. For example, the discriminative stimulus potencies of a series of agents might be related to their ability to activate a specific population of receptors (e.g., in tests of stimulus antagonism the effects can be effectively blocked by pretreatment of the animals with antagonists selective for this receptor population). Hence, structure–activity relationships can be formulated for their stimulus potencies as well as for their affinities for these receptors as measured by in vitro radioligand binding studies. It might be occasioned that an agent is identified that binds with high affinity, yet is behaviorally inactive; for example, the agent might be unable to penetrate the blood-brain barrier to activate the receptors of interest, or the agent is very rapidly metabolized in vivo. Hence, the structure–activity relationships would, at least initially, appear inconsistent. To distinguish between the two types of structure–activity relationships, the former are termed in vivo SAR whereas binding relationships are often referred to as in vitro SAR or, preferably, *structure–affinity relationships* (SAFIR). The opposite effect can also be encountered. For example, a member of the series of agents described above might be quite potent in tests of stimulus generalization, and its effects antagonized by the selective antagonists, but the agent lacks affinity for the identified receptor population. This type of agent might be a prodrug that lacks receptor affinity but is metabolized in vivo to an active agent. Alternatively, the agent might act through a somewhat different specific mechanism (see discussion of "general mechanistic similarity" and "specific mechanisms" in Chapter 6/section A). SAR studies are very useful when interpreted cautiously and conservatively, but "outlier" agents should be carefully re-examined rather than dismissed.

The several examples that follow are meant to be an illustrative rather than detailed accounting of SAR studies using drug discrimination techniques. One of the most comprehensive SAR studies using drug discrimination was that involving phenylalkylamines and, as such, is a major focus of this chapter. Additional examples will be described in subsequent chapters where results are used in examining mechanisms of drug action or for developing novel pharmacological tools.

B. PHENYLALKYLAMINE HALLUCINOGENS AND STIMULANTS

1. Phenylalkylamines

Structurally, phenylalkylamines (also sometimes referred to as *phenalkylamines*) are composed of an *aryl ring* separated from a terminal amine (most notably by a two-methylene unit chain). *Phenylisopropylamines* (PIAs) are a special case of phenylalkylamines where the aryl ring is separated from the terminal amine by a two-methylene unit and the carbon atom attached to the amine (i.e., the α carbon atom) bears a methyl (i.e., $–CH_3$) group. "*Phenylisopropylamines*" represent a family of pharmacologically active substances. And, the parent member of the phenylisopropylamine family is known as amphetamine or, simply, as phenylisopropylamine (see Figure 5-1). Replacement of the α-methyl (i.e., $–CH_3$) group of *phenylisopropylamines* with a hydrogen (–H) atom converts them to a group of compounds known as "*phenylethylamines*" (PEAs); the parent member of this class of agents is phenylethylamine itself (Figure 5-1). In brief, phenylalkylamines can be viewed as an umbrella group of agents that includes phenylisopropylamines and phenylethylamines. Structure-activity relationships for the general and varied pharmacological actions of phenylalkylamines have been reviewed [1–3].

One of the early SAR studies to focus on an extensive series of structurally related agents utilizing drug discrimination methodology involved rats trained to discriminate the phenylisopropylamine hallucinogen DOM (see Figure 5-1 for chemical structure) from vehicle. DOM is obtained by the incorporation of various aromatic (i.e., aryl) substituents on the phenylisopropylamine molecule. Another way of looking at this is

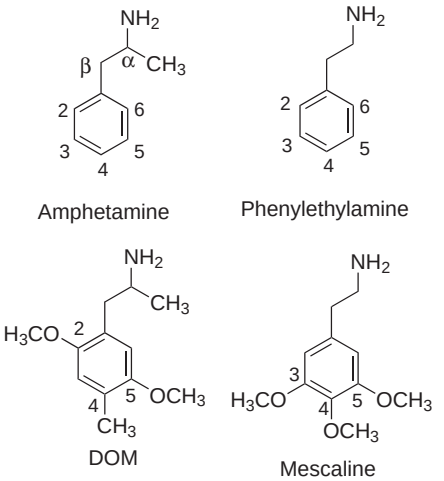


Figure 5-1. Chemical structures of representative phenylalkylamines: the phenylisopropylamines amphetamine and DOM, and the phenylethylamines phenylethylamine and mescaline.

that removal of all aromatic ring (i.e., "aryl") substituents from the hallucinogen DOM results in the central stimulant amphetamine. Both amphetamine and DOM are phenylisopropylamines (i.e., both have the same "*parent*" phenylisopropylamine structure). Evidently, "aryl" substituents dramatically influence the general pharmacology of these agents; that is, one of these phenylisopropylamines (i.e., amphetamine) is a central stimulant and one (i.e., DOM) is a classical hallucinogen. Consistent with this concept is that DOM-stimulus generalization, using (±)DOM-trained rats, does not occur to amphetamine or either of its optical isomers, nor does a (+)amphetamine-stimulus (using (+)amphetamine-trained rats) generalize to DOM, nor either of its optical isomers, despite the fact that both agents are phenylalkylamines and, more precisely, phenylisopropylamines [4].

The phenylalkylamine mescaline (see Figure 5-1 for chemical structure) is also a classical hallucinogen. However, rather than being a phenylisopropylamine, mescaline is a phenylethylamine. It might be noted here that mescaline is the phenylethylamine—or the α-*des*methyl—counterpart of the phenylisopropylamine 3,4,5-TMA (see Figure 5-3 to follow).

A logical approach to investigating the influence of the chemical structure of DOM on DOM-like stimulus action would be to "deconstruct" the DOM structure in a stepwise manner. That is, each aryl substituent of DOM could be individually removed, one at a time, to determine it's role on DOM-appropriate responding (i.e., to identify whether or not removal of that substituent—or, more precisely, replacement of the substituent by a hydrogen atom—results in stimulus generalization and, if it does, to determine how potent the new analog is relative to the parent). This is a general approach that we and others have employed over the years (i.e., the "*deconstruction-reconstruction-elaboration*" approach to drug design; see APPENDIX) in drug discrimination and other pharmacological studies. For example, what is the role of the DOM 4-methyl group? Does it contribute to action? to potency? Replacement of the methyl group with H affords 2,5-DMA (i.e., the 4-*des*methyl counterpart of DOM). In fact, 2,5-DMA (ED_{50} = 4.6 mg/kg) substitutes for DOM (ED_{50} = 0.45 mg/kg) in DOM-

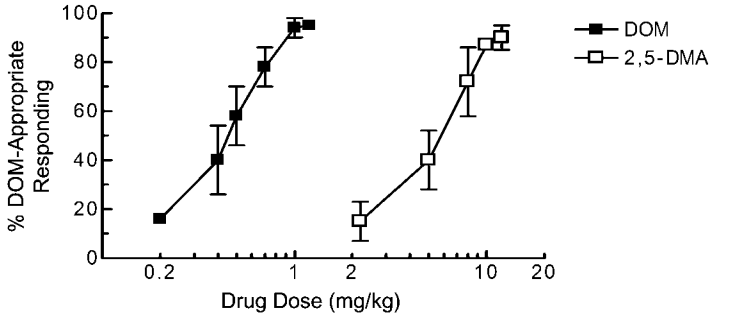


Figure 5-2. Results of stimulus generalization studies with DOM and its 4-desmethyl counterpart, 2,5-DMA, in rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle. Saline produced <20% DOM-appropriate responding.

trained rats but was 10-fold less potent than its parent (Figure 5-2) [4]. As a consequence, it seems that the methyl group contributes to potency but not necessarily the stimulus nature of DOM. However, we are getting ahead of the story. A somewhat different approach was employed for this particular series of studies.

Because it was of interest to determine the influence of phenylalkylamine aryl (and other) substituents both on "DOM-like" and "amphetamine-like" stimulus actions, and because certain phenylalkylamine hallucinogens (e.g., mescaline) possess a different aryl substitution pattern than DOM, it was thought more logical and expedient to begin with the parent aryl-unsubstituted phenylisopropylamine (i.e., amphetamine), and to introduce substituents in a stepwise fashion. These targets could then be examined both in (±)DOM- and (+)amphetamine-trained rats. This project required the synthesis of numerous target compounds and their optical isomers and involved stimulus generalization studies in two groups of (i.e., DOM- and (+)amphetamine-trained) animals; as a consequence, the studies were described in a series of related publications published over a period of several years. The basic question addressed was: how do aryl (and other) substituents influence the DOM-like and amphetamine-like nature of phenylalkylamines as discriminative stimuli? Findings with DOM-trained rats will be described first, followed by results obtained by an examination of these same agents in (+) amphetamine-trained animals. The findings have been reviewed [4–6]. Because the molecular weights of these agents did not substantially differ, ED_{50} values will be described (here) in terms of mg/kg. It might be noted, however, that where more strict potency comparisons were made, ED_{50} values were compared on a μmole/kg basis for greater accuracy [4, 5; see also Chapter 3].

2. DOM-Like SAR

Various methoxy-substituted analogs of the basic phenylisopropylamine structure were examined in rats trained to discriminate DOM (1.0 mg/kg) from saline vehicle. These included the three possible monomethoxy positional isomers: the 2-methoxy, 3-methoxy, and 4-methoxy analogs OMA, MMA, and PMA, respectively (see Figure 4-2 for chemical structures), and a number of di- and trimethoxy analogs (DMAs and TMAs, with preceding numbers indicating the position of the methoxy groups) (see Figure 5-3 for chemical structures). None of the monomethoxy compounds, that is: OMA, MMA, or PMA, produced greater than 20% DOM-appropriate responding at the highest non-disruption doses evaluated [7] indicating that they were *devoid* of DOM-like stimulus character at the doses examined and under the conditions employed. These agents are not necessarily devoid of central action; rather, the results simply suggested they are devoid of (i.e., different from) DOM-like stimulus actions at the doses and conditions at which they were assayed. There exist six positional isomers of the DMAs: 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-DMA. The DOM stimulus generalized only to 2,4-DMA and 2,5-DMA (ED_{50} = 4.88 and 5.80 mg/kg, respectively) (Figure 5-4), but neither agent was as potent as DOM (ED_{50} = 0.44 mg/kg) [7, 8]. Administration of 3,4-DMA elicited a maximum of 46% DOM-appropriate responding (at 9.0 mg/kg) and disrupted the animals' behavior following administration of higher doses (i.e., at 10.5 and 12 mg/kg) of drug (data not shown in Figure 5-4 for purpose of clarity of presentation). Of the

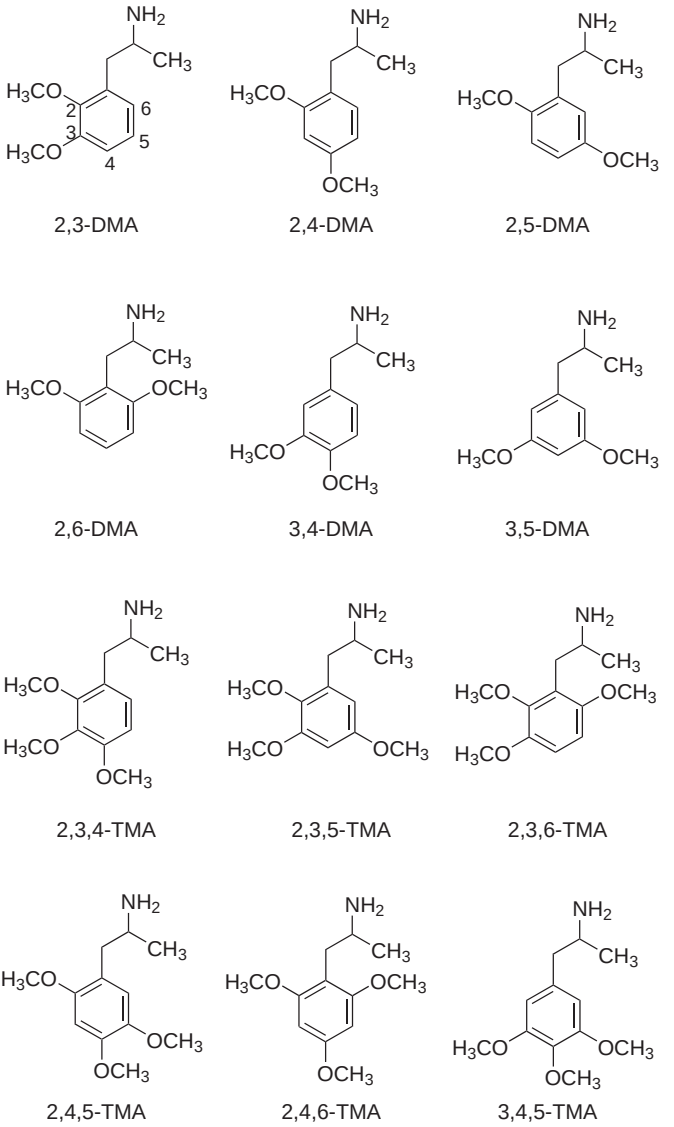


Figure 5-3. Chemical structures of the 12 possible di- and trimethoxy phenylisopropylamines (i.e., DMAs, and TMAs, respectively). Then "number designators" indicate the position of pendant methoxy groups.

six possible trimethoxy analogs (TMAs), five were examined (Figure 5-5), and the DOM stimulus generalized to all five: 2,3,4-TMA (ED_{50} = 7.80 mg/kg), 2,3,5-TMA (ED_{50} = 16.48 mg/kg), 2,4,5-TMA (ED_{50} = 3.59 mg/kg), 2,4,6-TMA (ED_{50} = 3.69 mg/kg), and 3,4,5-TMA (ED_{50} = 6.34 mg/kg). Here, too, all were substantially less potent than DOM [8].

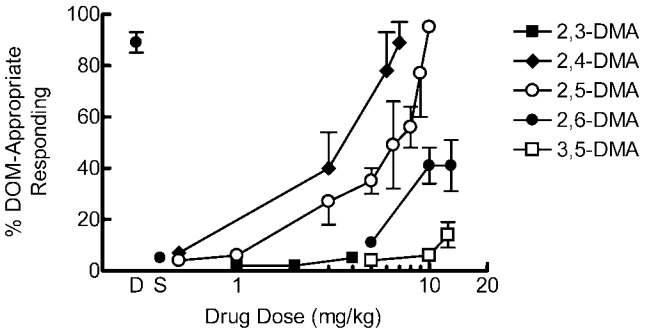


Figure 5-4. Results of stimulus generalization studies with DMA analogs using rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle [7, 8]. Administration of higher doses of 2,3-DMA, 2,6-DMA, and 3,5-DMA resulted in behavioral disruption. D = effect of the training dose of DOM; S = effect of saline.

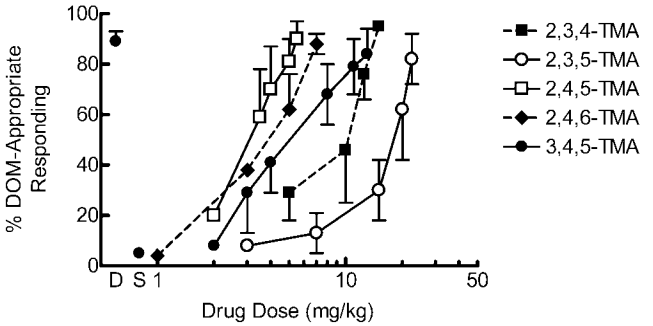


Figure 5-5. Results of stimulus generalization studies with TMA analogs using rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle [8]. D = effect of the training dose of DOM; S = effect of saline.

Apparently, the three monomethoxyphenylisopropylamines were "inactive" with regard to producing DOM-like stimulus actions under the conditions they were assayed, a 2,4- or 2,5-dimethoxy substitution pattern was optimal for the DMAs, either a 2,4,5- or 2,4,6-trimethoxy substitution pattern was optimal for the TMAs, and, in the case of the dimethoxy analog 2,5-DMA, the presence of an additional aryl methyl group (i.e., a 4-methyl group as found in DOM) resulted in enhanced potency. The study emphasized the importance of the phenylisopropylamine *2,5-dimethoxy substitution pattern* for producing DOM-like stimulus actions. It might be noted that 4-substituted 2,6-DMA analogs might also be interesting to investigate in stimulus generalization studies, but this has not yet been reported.

On the basis of the results just described, the presence of the 4-methyl group of DOM obviously contributes to the stimulus nature and potency of 2,5,-DMA as a DOM-

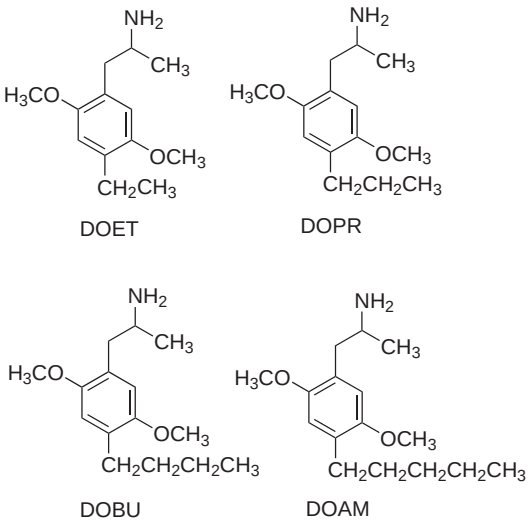


Figure 5-6. Chemical structures of the DOM-related homologs DOET, DOPR, DOBU, and DOAM.

like agent. *Homologation* (i.e., "extension" of an alkyl group by additional carbon or methylene units) of the 4-methyl group of DOM to an ethyl group results in DOET (Figure 5-6). In fact, the human psychoactive effects of DOM and DOET were first described in the same publication [9]. The stimulus nature of several DOM homologs were compared in DOM-trained rats, including those of DOET (the ethyl homolog of DOM), DOPR (the *n*-propyl homolog), DOBU (the *n*-butyl homolog), and DOAM (the *n*-pentyl or amyl homolog) [10]. DOM-stimulus generalization occurred only to the shorter-chain homologs (Figure 5-7): DOET (ED_{50} = 0.23 mg/kg) and DOPR (ED_{50} = 0.17 mg/kg) were somewhat more potent than DOM, whereas the four-carbon or *n*-butyl homolog DOBU (ED_{50} = 0.91 mg/kg) was somewhat less potent [7]. Administration of various doses of the five-carbon chain compound, DOAM, resulted only in partial generalization [7].

3. Amphetamine-Like SAR

Nearly all of these same compounds were examined in rats trained to discriminate (+) amphetamine (1.0 mg/kg) from saline vehicle and, in short, the results were nearly mutually exclusive. In general, phenylisopropylamines that displayed DOM-like character failed to fully substitute for (+)amphetamine—there were a few exceptions (e.g., see Figure 4-22), and these will be discussed in detail in Chapter 6. The (+)amphetamine stimulus failed to completely generalize to phenylisopropylamines bearing multiple aryl methoxy substituents. No di- or trimethoxyphenylisopropylamine (i.e., DMA or TMA analog) produced (+)amphetamine-like stimulus effects; in contrast, three agents (i.e.,

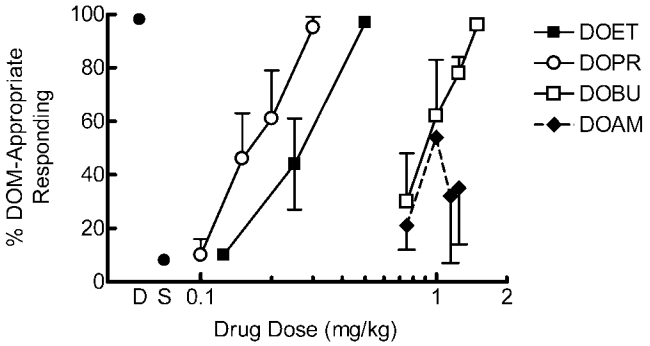


Figure 5-7. Results of stimulus generalization studies with DOM homologs using rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle [10]. A dose of DOAM > 1.25 mg/kg disrupted the animals' behavior. D = effect of the training dose of DOM; S = effect of saline.

positional isomers) that failed to produce DOM-appropriate responding in DOM-trained animals substituted in the (+)amphetamine-trained rats: OMA, MMA, and PMA (see Chapter 4/section A). Furthermore, because dimethoxy analogs failed to substitute, the 2- and 5-methoxy groups of DOM were removed to afford (±)4-methylamphetamine (racemic *p*TAP; see Figure 4-1); this agent also failed to substitute in the (+) amphetamine-trained animals (see also Chapter 6 for mechanistic discussion).

4. SAR Comparisons

As demonstrated above, structure-activity relationships for eliciting DOM-like *versus* (+)amphetamine-like stimulus effects were quite different (but, see Figure 4-22 and discussion of MDA in Chapter 6) despite a common phenylalkylamine structure. Other differences exist. It is known that *N*-monomethylation enhances the stimulant and discriminative stimulus potency of amphetamine (i.e., methamphetamine; Figure 4-1). What is the effect of *N*-methylation of DOM-related compounds? Will this molecular modification convert a hallucinogen to a central stimulant? In DOM-trained rats, *N*-monomethyl DOM (ED_{50} = 3.99 mg/kg) was one-tenth as potent as DOM, whereas *N*,*N*-dimethyl DOM was even less potent (ED_{50} = 5.37 mg/kg) [11]. Neither agent substituted in (+)amphetamine-trained rats. Here is another example of a clear difference in the structural requirements for phenylalkylamines to produce amphetamine-like *versus* DOM-like stimulus effects. Yet another is the optical activity of the phenylisopropylamines. For DOM-like actions, *R*-isomers are more potent than their *S*-isomers, whereas the reverse is true for those agents that produced amphetamine-like effects. Nevertheless, the actions of these agents are generally *stereoselective* (or *enantioselective*); that is, both isomers are active, (i.e., one isomer is more potent than the other). But, similarities also exist. For example, replacement of the α-methyl group of a phenylisopropylamine by a hydrogen (i.e., H) atom, to convert it to a phenylethylamine,

typically resulted in reduced potency. Mescaline (see Figure 5-1 for chemical structure), as mentioned above, is the α-*des*methyl counterpart of 3,4,5-TMA (see Figure 5-3 for chemical structure); both agents substituted in DOM-trained rats with mescaline (ED_{50} = 14.6 mg/kg) being about four times less potent than 3,4,5-TMA (ED_{50} = 3.7 mg/kg) on a mg/kg or μmole/kg basis [8]. Likewise, DM-DOM, the α-desmethyl or phenylethylamine counterpart of DOM, produced DOM-like stimulus effects (ED_{50} = 1.3 mg/kg) but was about 3-fold less potent than DOM [11]. As a general rule with respect to DOM-like stimulus generalization potency, and human hallucinogenic potency, phenylethylamines are typically three to four times less potent than their corresponding phenylisopropylamines. The same structural change converts amphetamine to phenylethylamine (see Figure 5-1 for chemical structures); although a (+)amphetamine stimulus generalized to racemic amphetamine (ED_{50} = 0.62 mg/kg), administration of six doses of phenylethylamine ranging from 1.0 to 5.75 mg/kg produced <20% drug-appropriate responding [12]. Examination of higher doses was precluded by behavioral disruption. Thus, phenylethylamine, should it be an amphetamine-like agent, would be expected to be >10-fold less potent than amphetamine. The inactivity of, or lack of (+) amphetamine-stimulus generalization to, phenylethylamine was attributed to the difficulty of the latter to penetrate the blood-brain barrier and, should a small amount enter the brain, to its rapid metabolism by monoamine oxidase [12].

In this manner, it was possible to determine how various structural features of phenylalkylamines contribute to DOM-like stimulus effects and what substituents contribute to (+)amphetamine-like stimulus effects; see Figure 5-8 for a brief SAR summary and comparison of the structural requirements optimal for the two types of effects.

This was, perhaps, the first time that various agents belonging to a common structural class (i.e., phenylalkylamines) had been categorized as producing one type of stimulus effect over another, and the SAR for producing the two different stimulus effects was delineated. A salient feature of these investigations was that drug discrimination studies, in tests of stimulus generalization, can be employed to *categorize* or *classify* the stimulus effects produced by agents that are closely related in chemical structure. Hence, these types of studies, in addition to providing general SAR data

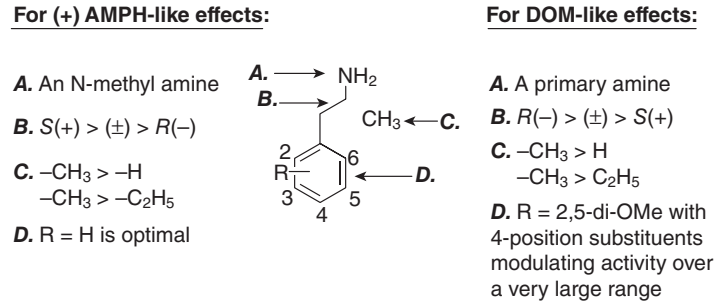


Figure 5-8. A general SAR summary of the effect of structural modification on amphetamine-like and DOM-like stimulus actions.

(useful for other applications—as will be described later), were used to obtain information about the "classification" of the stimulus effects produced by structurally-related agents. Another ramification of these studies was that *agents that bear close structural similarity to one another might not produce common stimulus effects in animals*. This will be further discussed in Chapter 6.

5. QSAR Investigations

Quantitative structure–activity relationship (QSAR) *studies* are a step beyond SAR studies. SAR (and SAFIR) studies are aimed at determining what and which structural features (i.e., "substituents" or "substituent groups") of a molecule, or of a related series of molecules, are important for, or influence, a particular pharmacological action and/or potency. They seek to identify which substituents groups are important or unimportant, and at what specific molecular location such substituents impact action or potency. In contrast, QSAR studies strive to identify how or why these various substituent groups influence potency. As its name implies, QSAR results are quantitative rather than qualitative. QSAR studies aim to derive specific, quantitative mathematical relationships between potency and chemical character. The focus is on the physicochemical properties associated with a given substituent in a molecule(s) of interest (i.e., specific atoms, functional groups and, sometimes, molecules as a whole). Many different techniques are available to medicinal chemists for investigation of QSAR. Questions that can be asked include: is it the lipophilic (i.e., hydrophobic), steric (e.g., volume, length, width, shape, size), electronic (electron withdrawing, electron donating), hydrogen bonding (i.e., hydrogen bond acceptor, hydrogen bond donor) nature, or some other property, of a substituent(s) that best explains the actions of a molecule? The lipophilic (or hydrophobic) nature of substituent atoms/groups is reflected by their π values, whereas their electronic effects can be expressed as Hammett σ values. Steric and shape properties can be investigated using any of several different substituent parameters including, for example, Taft steric indicies (E_s), Kier-Hall shape indicies (κ), or Verloop STERIMOL parameters. Many other properties (or "*physicochemical parameters*") can be examined. By comparing the actions (i.e., potencies) of a series of agents with these substituent parameters, it is often possible to identify a correlation between potency (i.e., the dependent variable) and one or more of these factors (i.e., the independent variable(s)) by conducting a Hansch analysis or by using some other related technique. Hansch analysis has its roots in linear free energy relationships and can be defined as Log 1/C = mx + b (where C = molar drug concentration) and x is a particular physicochemical parameter. The original Hansch calculations assumed Log $1/C = k_1\pi + k_2\sigma + k_3$ (where k are constants). Other parameters have since been introduced and incorporated to extend the analysis. For further discussion of the general approach, see Burger's Medicinal Chemistry [13].

QSAR analysis is particularly useful for detailed investigations of in vitro data, but is far less frequently employed for evaluation of in vivo data because the actions of drugs in vivo are associated with absorption, distribution, and metabolic factors that can often confound interpretation of any resulting *relating equations*. Nevertheless, because a number of phenylisopropylamines had been shown to produce a common

DOM-like stimulus effect in rats, it was of interest to see if QSAR studies might implicate specific molecular substituents responsible for this action. A preliminary Hansch analysis was conducted on the generalization potencies [5] of a series of 4-substituted 2,5-DMA analogs to substitute in DOM-trained rats (as already discussed above, the 2,5-dimethoxy substitution pattern of DOM-like phenylisopropylamines was found important, and variation of the 4-position substituent was shown to influence potency). In this series of agents, structural variation was limited only to a single position (i.e., the 4-position of 2,5-DMA). For a series of 2,5-DMA analogs varying only in the nature of their 4-position substituent, a relating equation was obtained indicating that DOM-stimulus potency was related to the lipophilicity (i.e., π value) of the 4-position substituent (i.e., Log 1/C = $2.28\pi - 0.94\pi^2 + 4.81$; n = 11, r = 0.977, F-ratio = 86.5). The relationship was parabolic. That is, potency increased as the lipophilicity of the 4-position substituent increased (indicated by the positive correlation with π), but decreased as substituent lipophilicity increased beyond a certain point (i.e., as indicated by the π^2 term in the relating equation). What this implied was that the lipophilicity of the 4-position substituents of 2,5-DMA analogs contributed to their DOM-like stimulus potency, but that as lipophilicity continued to increase, potency decreased. Using the relating equation, the actual DOM-stimulus generalization potencies of these agents were plotted against their ED_{50} values calculated by the relating equation (Figure 5-9). The relating equation seemed able to explain the potencies of these agents. Furthermore, the results correctly predicted the decreased potencies of certain DOM homologs (e.g., the 4-isopropyl and 4-*n*-butyl analogs of 2,5-DMA: DOIP and DOBU, respectively) and the lack of DOM-stimulus generalization to DOAM [5]. Also consistent was that compounds bearing polar 4-position substituents (i.e., substituents that contribute to reduced lipophilicity) such as those found in DOOC and DOOH (i.e., the

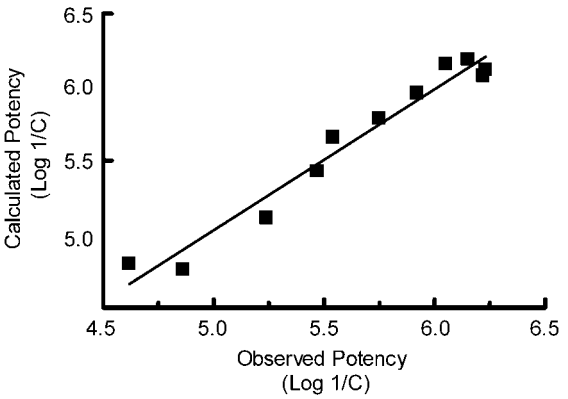


Figure 5-9. Observed versus calculated potencies (Log 1/C where C is the ED_{50} dose for DOM-stimulus generalization in moles/kg) for a series of 2,5-DMA analogs based on a relating equation identified by Hansch analysis utilizing π and π^2 terms (n = 11; r = 0.977). The analogs included 2,5-DMA and 10 of its 4-substituted derivatives: -OMe, -F, -nBu, -iPr, -Me (DOM), -Cl, -Et, -I, -Br, -nPr.

carboxy and 4-hydroxy analogs of 2,5-DMA) failed to substitute for DOM in DOM-trained animals. Although the relating equation nicely accounted for the drug discrimination findings, little information of mechanistic value was gained. That is, DOOC and DOOH might not bind at the receptors activated by DOM, or these latter compounds are simply too polar to penetrate the blood-brain barrier to access the site of action of DOM. Questions remained. Once it was identified that DOM behaved as a 5-HT_2 receptor agonist, QSAR studies continued, but they focused on the 5-HT_2 receptor affinities of these and related agents rather than on their stimulus generalization potencies [14, 15] (see Chapter 7 for continued discussion of this topic). Nevertheless, this was the first time QSAR studies had been performed using drug discrimination (i.e., stimulus generalization) data.

C. BENZODIAZEPINES

1,4-Benzodiazepines, subsequently referred to here simply as "benzodiazepines," such as chlordiazepoxide (Librium®) and diazepam (Valium®), represented the first members of a class of therapeutic agents shown to produce a specific antianxiety or anxiolytic effect in humans. At one time, chlordiazepoxide and diazepam were the two most widely prescribed drugs in the United States. Benzodiazepines, depending upon their substitution pattern (Figure 5-10), also produce varying degrees of muscle relaxant, sedative, anticonvulsant, and/or other actions. A study was undertaken 25 years ago to examine the SAR of various benzodiazepine analogs to produce diazepam-like discriminative stimulus effects. Are the stimulus effects of benzodiazepines related? Are they related to the anxiolytic effects of the benzodiazepines? Do metabolites of the benzodiazepine anxiolytics contribute to their stimulus effects? In other words, are benzodiazepine metabolites capable of producing diazepam-like stimulus actions? What is the general SAR for producing diazepam-like stimulus effects in animals? It was possible to address these questions using rats trained to discriminate the benzodiazepine anxiolytic agent diazepam from saline vehicle.

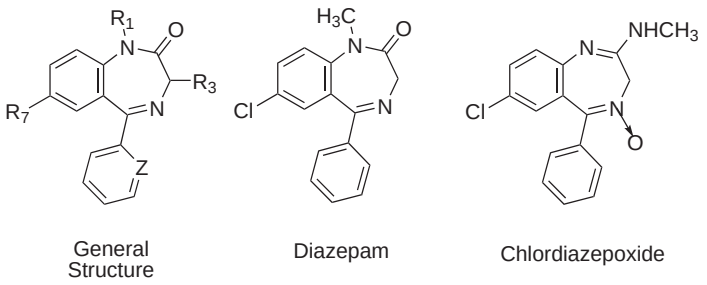
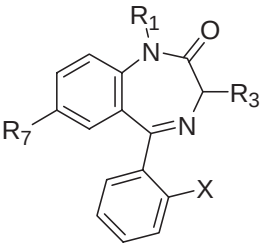


Figure 5-10. General chemical structure of 1,4-benzodiazepine anxiolytic agents. Z = N or CH-X where X is H, halogen, or a CF_3 group (see text for further explanation), and two specific examples: diazepam and chlordiazepoxide.

TABLE 5-1. Representative results of substitution studies with various benzodiazepines using rats trained to discriminate 3.0 mg/kg (IP) of the anxiolytic agent diazepam from saline vehicle



Agent	R_1	R_3	R_7	X	ED_{50} (mg/kg)
Diazepam	$-CH_3$	H	Cl	H	1.22
Temazepam	$-CH_3$	-OH	Cl	H	1.54
Norazepam	H	H	Cl	H	2.35
Ro 5-2904	H	H	CF_3	H	0.47
Nitrazepam	H	H	NO_2	H	0.61
3-Me Flunitrazepam	$-CH_3$	$-CH_3$	NO_2	F	0.62
Clonazepam	H	H	NO_2	Cl	0.10
Ro 5-3027	H	H	Cl	Cl	0.12
Ro 5-3590	H	H	NO_2	CF_3	0.22

See [16] and Chapter 3 for further details.

A series of benzodiazepines (data for some of which are shown in Table 5-1) was examined in rats trained to discriminate 3.0 mg/kg of diazepam from saline vehicle [16]. Many of the benzodiazepine analogs examined substituted for the diazepam stimulus. Potencies varied over a very broad range. The results allowed formulation of in vivo or diazepam-like discriminative stimulus SAR. Some of the general conclusions were that 1) the N_1-methyl substituent of diazepam was not required for activity, 2) substituents at the C_3-position generally detract from (or abolish) activity/potency (depending upon the nature or stereochemistry of the substituent; see also Chapter 4), 3) substitution at the 2-position of the C_5-phenyl ring (i.e., the 2'-position) can moderate potency (depending upon its nature), and that 4) electron withdrawing groups at the benzodiazepine 7-position are important for diazepam-like actions. Ring-fusion also modulated action. A brief summary of the results obtained with several selected agents is shown in Table 5-1.

These studies not only contributed to an early understanding of diazepam-like stimulus effects but also enhanced understanding of diazepam-like stimulus mechanisms (see Chapters 3 and 6). All results were consistent, or at least were not inconsistent, with results obtained from human studies and suggested that certain "active"

benzodiazepine metabolites contribute to (i.e., might increase) the duration of action of the effect of administered benzodiazepine anxiolytic agents.

D. NEURONAL NICOTINIC ACETYLCHOLINERGIC RECEPTOR AGENTS

In the mid-1990s, newly available radioligands for labeling neuronal nicotinic acetylcholinergic (nACh) receptors caused a shift in research focus from long-time investigations of peripheral (i.e., muscle-type) nACh receptors using isolated tissue preparations to an investigation of neuronal nACh receptors using, primarily, radioligand binding studies. Early indications were that what was known about the SAR of nicotinic agents at peripheral nACh receptors would likely be different for neuronal nACh receptors. Research with neuronal nACh receptor agents needed to start from "scratch." Using a standard "*deconstruction*" approach (see APPENDIX), various simplified analogs of nicotine (see Figure 5-11 for chemical structure)—the prototypic nACh receptor ligand—were examined to determine the importance of various structural features on, and the minimal structural requirements for, the binding and actions of nicotine. In addition to receptor binding assays, initially conducted with [^{3}H]nicotine and later with [^{3}H](-)nicotine when it became available, nicotine analogs were also examined in other functional assays where nicotine was known to be pharmacologically active, including: spontaneous activity (mice), antinociceptive actions (mice), and drug discrimination studies (rats) using (-)nicotine as training drug. Not every analog was examined in every assay; analogs lacking nACh receptor affinity and that were shown to be inactive in preliminary behavioral assays were not typically examined in the more labor-intensive drug discrimination studies. Nevertheless, these deconstruction studies examined the influence of the pyridine nitrogen atom, stereochemistry, *N*-demethylation, the basicity of the pyrrolidine amine, and opening of the pyrrolidine ring of nicotine on nACh receptor affinity, and in selected functional studies. Of the structural modifications

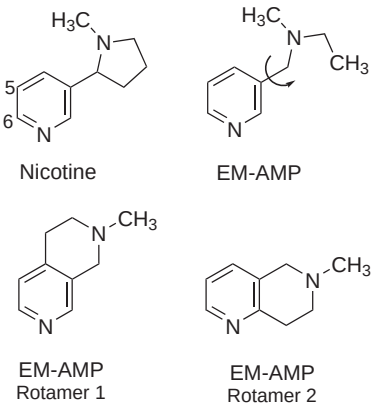


Figure 5-11. Chemical structures of the nACh receptor agonist ligand nicotine, EM-AMP, and two extreme conformationally constrained rotamers of EM-AMP.

examined, the intact (-)nicotine structure remained optimal. With regard to ring-opening, the nicotine partial-structure 3-(*N*-ethyl-*N*-methylaminomethyl)pyridine (EM-AMP; Figure 5-11) (K_i = 28 nM, relative to 2 nM for (-)nicotine), was the only compound to bind at nACh receptors with a K_i value of <100 nM. The corresponding *N*,*N*-dimethyl compound showed reduced affinity (K_i = 540 nM) whereas homologation of the ethyl group of EM-AMP to an *n*-propyl group, that is, EP-AMP (structure not shown; K_i = 1,140 nM) resulted in an even lower affinity.

In tests of stimulus generalization employing rats trained to discriminate 0.4 mg/kg of (-)nicotine from saline vehicle, EM-AMP produced a maximum of 49% drug-appropriate responding, and the *n*-propyl homolog produced a maximum of only 9% drug-appropriate responding (i.e., saline-like effects) [17]. EM-AMP can exist as rotamers (see arrow in Figure 5-11); two conformational extremes are represented by conformationally-constrained analogs of EM-AMP termed "Rotamer 1" and "Rotamer 2". EM-AMP Rotamer 2 displayed lower nACh receptor affinity (K_i = 165 nM) than EM-AMP and failed to produce nicotine-like actions in any of the behavioral assays. In contrast, EM-AMP Rotamer 1 displayed higher affinity (K_i = 18 nM), was active in the behavioral assays, and substituted (ED_{50} = 5 mg/kg) for (-)nicotine in tests of stimulus generalization [17]. The results of such studies served as the basis for a number of other investigations that led to the development of several novel nACh receptor agonists and antagonists. For example, the structural similarity between nicotine and the natural product epibatidine led to the synthesis of 6-chloronicotine [18] as one of the first nicotine analogs to bind with higher affinity at nACh receptors than nicotine. 6-Chloronicotine was six to fifteen times more potent than nicotine in behavioral assays [17] and a 6-chloropyridine ring is now common to many newer nACh receptor ligands. In another study, 5-methoxynicotine, was identified as an antagonist of certain actions of nicotine (i.e., antinociceptive effects) but not others (discriminative stimulus), whereas 5-bromonicotine was demonstrated to produce (-)nicotine-like stimulus effects [19].

This is an instance where drug discrimination studies were used in combination with radioligand binding and various other behavioral studies to optimize the actions of a particular agent (i.e., nicotine). The use of the drug discrimination paradigm aided these studies and assisted in the eventual development of several novel neuronal nACh receptor agonists and antagonists [reviewed: 20, 21].

E. AMINOTETRALINS

In 1984, 8-OH DPAT (see Figure 5-12 for chemical structure) was identified as a structurally novel 5-HT receptor agonist. An in vitro SAR investigation revealed that the *N*,*N*-di-*n*-propyl substituents of 8-OH DPAT were seemingly optimal for agonist action, and that 8-OH DPAT was essentially equipotent with its shorter-chain *N*,*N*-diethyl analog (8-OH DEAT), about seven times more potent than its *N*,*N*-dimethyl counterpart (8-OH DMAT), and >100 times more potent than its *N*,*N*-di-*n*-butyl homolog 8-OH DBAT [22].

Subsequently, rats were trained to discriminate 0.1 mg/kg of the serotonin receptor agonist 8-OH DPAT from saline vehicle, and it was demonstrated that the 8-OH DPAT

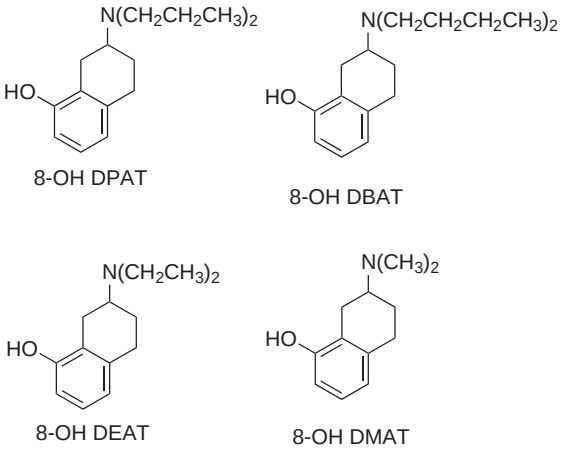


Figure 5-12. Chemical structure of 8-OH DPAT and its longer and shorter chain homologs.

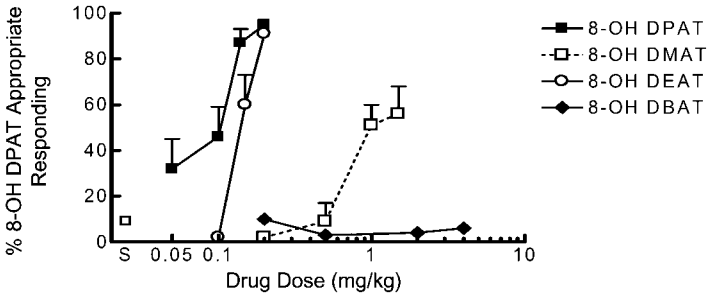


Figure 5-13. Results of stimulus generalization studies with 8-OH DPAT homologs (see Figure 5-12 for chemical structures) in rats trained to discriminate 0.1 mg/kg of 8-OH DPAT from vehicle. S = effect of saline administration. Administration of 8-OH DMAT doses greater than those shown resulted in behavioral disruption.

stimulus (ED_{50} = 0.08 mg/kg) potently generalized to 8-OH DEAT (ED_{50} = 0.13 mg/kg), but not to 8-OH DMAT or 8-OH DBAT (Figure 5-13) [23]. 8-OH DMAT produced a maximum of 56% 8-OH DPAT-appropriate responding (at 1.5 mg/kg) followed, at slightly higher doses, by disruption of the animals' behavior. 8-OH DBAT produced saline-like responding at up to 50 times the ED_{50} dose of 8-OH DPAT. The results of the drug discrimination studies were in general agreement with results from in vitro studies in that 8-OH DPAT was optimal, that the *N*,*N*-di-*n*-propyl groups could be replaced by *N*,*N*-diethyl groups, and that both of these agents were more effective than 8-OH DMAT and 8-OH DBAT in producing 8-OH DPAT-like effects.

More will be said about 8-OH DPAT analogs in Chapter 7.

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6

DRUG DISCRIMINATION AND MECHANISMS OF DRUG ACTION

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

A. EARLY CONSIDERATIONS

How/why do drugs exert stimulus control of behavior? What underlies the actions of an agent to serve as a discriminative stimulus in animals? Why can animals be trained to discriminate (i.e., recognize) certain agents from saline vehicle but not others? Why does stimulus generalization occur? These were some of the questions at the heart of drug discrimination and stimulus generalization studies in the formative years of this paradigm. Thinking evolved over time. At first it was thought that stimulus control of behavior might be related to the relative drug-induced response rates of animals and that this was associated with the ability of an agent to depress the rate at which an animal responds. If a "test agent" produced an alteration (e.g., reduction) in the animal's response rate, it was thought that the agent might be producing an effect similar to that of the training drug. It was also once thought that, perhaps, only drugs of abuse might serve as discriminative stimuli, and that the drug discrimination procedure might be useful for "*discriminating*" or distinguishing drugs of abuse from agents with little to no abuse liability. But, in retrospect, many of the early studies focused primarily on drugs of abuse, and they sometimes employed what might now be considered "stout" doses of training drugs, or test agents in tests of stimulus generalization. These high drug doses had a profound effect on animal response rates. Subsequently, it was realized that animals could be trained to discriminate substantially lower drug doses of these training agents and, in particular, much lower doses of certain abused substances. In fact, it is now recognized that the effect of training doses of training drugs on animals' response rates do not differ from their response rates observed following administration of saline vehicle (see Chapter 3). It should also be noted that different (relatively low) training doses of the same training drug (i.e., doses that produce saline-like response rates) might produce their stimulus effects via different mechanisms (e.g., see discussion of 5-OMe DMT in Chapter 1 and Chapter 3). So, as a general explanation, this argument gradually, but eventually, fell into disfavor.

Perhaps stimulus control of responding is related to the subjective effects produced by a drug—a proposition long-held, and still held, by some investigators. That is, it was once thought that agents belonging to a particular pharmacological class might produce a common stimulus effect in animals. Phencyclidine, (+)LSD, and Δ^9-tetrahydrocannabinol (THC) are classified by Hollister [1] as being psychotomimetic "*hallucinogens*." In theory, then, animals trained to discriminate one of these three agents should recognize the other two in tests of stimulus generalization. After all, all three were initially classified as hallucinogenic agents and *supposedly* produce similar subjective effects. This was not found to be the case [reviewed: 2]. Indeed, these three agents produce different effects in humans, and comparable results were obtained from stimulus generalization studies using animals trained to discriminate each of these agents from saline vehicle [reviewed: 2]. That is, stimulus generalization failed to occur upon administration of these agents to animals trained to discriminate the other two. Clearly, the stimulus effects of "hallucinogens" (or "psychotomimetics" as initially defined by Hollister [1]) are not identical.

If a stimulus is related to subjective effects, animals trained to discriminate the anxiolytic agent diazepam from saline vehicle should, according to this concept, rec-

ognize (i.e., a diazepam stimulus should generalize to) other agents that produce an anxiolytic effect. This, too, was not found to be the case [3; see also Chapter 3]. Animals trained to discriminate the anxiolytic diazepam from saline vehicle recognized other benzodiazepine anxiolytics, but failed to recognize the novel (at that time) anxiolytic agent buspirone. More recently, it has been demonstrated that animals trained to discriminate certain antidepressants fail to generalize to certain other antidepressants (e.g., see [4] and [5]).

So, as suggested by a growing body of evidence, the *subjective effects produced by an agent might not be responsible for stimulus control of responding*, although, it should be noted that this concept might still hold true for certain types of agents—this remains to be fully investigated (e.g., see Chapter 16 by Colpaert).

A turning point came in the very late 1970s. Perhaps the stimulus effects of drugs are related to their mechanism of action rather than to the overt subjective effects they might produce. There are several means to test this hypothesis: 1) if an agent produces its stimulus effects via a specific receptor agonist-mediated mechanism, stimulus generalization might occur to agents that are selective agonists for that receptor type; 2) the stimulus effects of such an agent might be attenuated by antagonists that are selective for that receptor type; and 3) there might exist a relationship between the stimulus potencies of agents that substitute for the training drug and their affinities for a specific neurotransmitter receptor (or transporter, etc…). Although these concepts might have been considered ground-breaking in the field of drug discrimination studies at the time, this now has been demonstrated again and again, and several examples will be provided. However, such studies cannot always be taken at simple face value and might require further investigation. Consider an agent that releases a particular neurotransmitter, and an agent that blocks that neurotransmitter's reuptake. These two agents might produce a common stimulus effect by increasing synaptic levels of a specific neurotransmitter to, thereby, provide evidence for *general mechanistic similarity* (i.e., that a particular neurotransmitter is involved in the actions of the two agents). Yet, as defined by the example, the two agents increase synaptic concentrations of the neurotransmitter by different *specific mechanisms*: one agent acts as a releasing agent and the other as a reuptake inhibitor. A third agent might also produce a similar stimulus effect by direct activation of postsynaptic receptors—receptors that were activated by increased synaptic levels of neurotransmitter in the preceding example. And a fourth example can be encountered upon administration of allosteric agents that indirectly activate the receptor of interest. A problem, then, might be encountered with novel agents when it is not known *a priori* whether the agent is a releasing agent, reuptake inhibitor, direct receptor agonist, or allosteric activator. Evidence for "*general mechanistic similarity*" might be supported, but it is necessary to conduct additional studies to sort through the various possibilities to identify a "*specific mechanism*" over a "*general mechanism*." A very salient example of this concept is diazepam-stimulus generalization to pentobarbital, and pentobarbital-stimulus generalization to diazepam; these agents seem to produce similar stimulus effects and result in cross-generalization when each is used as training drug (see Chapter 3), but their specific mechanisms of action have been shown to differ. Both these agents are capable of modulating GABA-ergic neurotransmission, but do so by interaction at different *allosteric* binding

sites on $GABA_A$ receptors. This was described in Chapter 3. Other examples will be provided below.

Certain agents (e.g., opioids, amphetamine) were already known to produce at least some of their pharmacological effects *via* a direct or indirect receptor-mediated mechanism (opioid and dopaminergic receptor mechanisms, respectively). Consequently, it was logical to determine whether the same was true for their discriminative stimulus effects. Indeed, opioid receptor antagonists were shown to antagonize the stimulus effects of opioids, and dopamine receptor antagonists were demonstrated to antagonize the stimulus effects of amphetamine. These were gratifying results, but not unexpected findings. There are, however, instances where the mechanism of action of a substance, or a group of substances, was unknown and where drug discrimination studies aided in defining their underlying mechanisms. Several of many representative examples will be described.

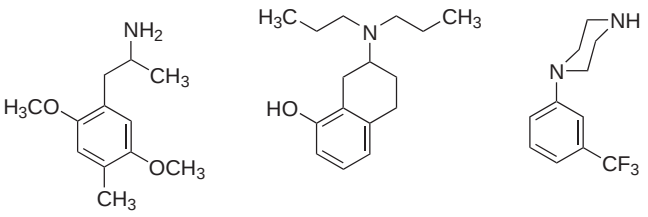
It should be appreciated that examples described in this chapter are primarily those generated from our laboratories. They represent only a small number of examples from the overall general drug discrimination literature. Different authors could have just as readily selected other examples. The interested reader is urged to consult the proceedings of several past drug discrimination symposia for additional examples [e.g., 6–9].

B. CLASSICAL HALLUCINOGENS

1. Historical Perspective

It was thought that the stimulus potencies of "*classical hallucinogens*," a subset of the larger group of agents termed "psychotomimetics" by Hollister [1], to produce a discriminative stimulus effect in animals might be related to their ability to bind at, and activate, a particular type of neurotransmitter (i.e., serotonin; 5-HT) receptor [2]. At the time, this was a novel concept. It was not a great leap in faith to speculate that indolealkylamine hallucinogens might act through a mechanism involving the structurally similar indolealkylamine neurotransmitter serotonin. Structurally, however, phenylalkylamine hallucinogens bear a much greater similarity to the phenylalkylamine neurotransmitters dopamine, epinephrine, and norepinephrine than they do to serotonin. For many years, it was thought, and quite reasonably so from a structural perspective, that phenylalkylamine hallucinogens might act through a dopaminergic and/or (nor) adrenergic mechanism. Yet, there were no published mechanistic reports to support this idea. A series of studies later supported the conclusion that "*classical hallucinogens*" likely act through a serotonergic mechanism regardless of their structural similarity to serotonin, dopamine, epinephrine, or norepinephrine [10–13]. Finally, the "great mystery" was solved. Animals, in general, might recognize the activation of a particular neurotransmitter receptor system, at least in some cases, to produce their stimulus effects. Specifically, it was reported that the stimulus effects of classical hallucinogens involved the activation of central serotonin receptors [13].

As happenstance would have it, there were a number of questions and discoveries over the ensuing years that argued against this nascent conclusion. First, the serotonin



DOM 8-OH DPAT TFMPP

Figure 6-1. Chemical structures of several early 5-HT receptor agonists.

receptors at which these hallucinogens had been initially examined were those found in a peripheral (rat fundus) tissue preparation, and the relevance of these peripheral 5-HT receptors to central (i.e., brain) 5-HT receptors was unknown at that time. Second, two novel and structurally distinct, 5-HT receptor agonists were introduced in the 1980s: 8-hydroxy-(2-di-*n*-propylamino)tetralin (8-OH DPAT) by a Swedish group at Uppsala University and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) by investigators at Eli Lilly (Figure 6-1). In theory, if hallucinogens were acting *via* a serotonin receptor agonist mechanism, animals trained to discriminate a classical hallucinogen, such as DOM, should recognize these novel agonists. As it turned out, animals trained to discriminate the "purported 5-HT receptor agonist" hallucinogen DOM did not recognize (i.e., DOM-stimulus generalization did not occur to) either 8-OH DPAT nor to TFMPP. Do the classical hallucinogens, perhaps, work through a non-serotonergic mechanism? Arguing against this, the DOM-stimulus generalized to the 5-HT-releasing agent fenfluramine [14] supporting a general mechanistic role for serotonin. (Dismissing whether or not it is related to the current argument, it might be parenthetically noted that ingestion of high doses of fenfluramine have been reported to produce hallucinogenic effects in humans similar to those of "LSD and certain ring-substituted [phenylisopropylamines]" [15].) Was it possible that 8-OH DPAT and TFMPP failed to substitute because they are unable to penetrate the blood-brain barrier? The subsequent use of 8-OH DPAT and TFMPP as training drugs demonstrated that each of the three agents produced a unique discriminative stimulus effect [reviewed: 14]. That is, using each of these three purported 5-HT receptor agonists, DOM, 8-OH DPAT, and TFMPP as training drugs, animals in each training group failed to recognize the other two purported 5-HT receptor agonists! Of course this did not constitute "*proof*" that 8-OH DPAT and TFMPP can penetrate the blood-brain barrier to produce their stimulus effects via a central serotonergic mechanism but, as described in Chapter 3, it is highly uncommon for peripherally acting agents to serve as training drugs in rats. And, there is nothing about the chemical structure of TFMPP to suggest it should not penetrate the blood-brain barrier (i.e., it might be argued that the presence of the hydroxyl group of 8-OH DPAT might reduce its lipophilicity and intrinsic ability to enter the brain—but, this was later shown not to be the case). Thus, it would seem quite unlikely (or highly coincidental) that both 8-OH DPAT and TFMPP produce their stimulus effects via a peripheral cue (and, if they did, there was certainly no reason why DOM could not act peripherally as well as centrally). Perhaps the answer to the problem was more complex

than simple activation of serotonin receptors. This raised the possibility of *multiple 5-HT receptor types* in the brain, just as multiple 5-HT receptor types (i.e., D and M) were already known to exist in the periphery. Indeed, at nearly the same time these studies were conducted, Peroutka and Snyder [16] identified two different serotonin receptor subtypes in brain tissue homogenates: 5-HT_1 and 5-HT_2 receptors. Later, 5-HT_3 receptors were described. Today, at least seven different populations of 5-HT receptors are recognized: 5-HT_1—5-HT_7 receptors [3].

2. Evidence for 5-HT_2 Receptor Involvement

Perhaps classical hallucinogens function by activating a particular population of 5-HT receptors to which the newer 5-HT receptor agonists 8-OH DPAT and TFMPP did not show high affinity and/or agonist action. Indeed, there was a significant correlation between the stimulus generalization potency in DOM-trained animals and the 5-HT_2 receptor affinities of these agents [reviewed: 17]. Furthermore, neither 8-OH DPAT nor TFMPP displayed high affinity for this receptor population. Also, the stimulus effects of classical hallucinogens were antagonized by the (then) newly discovered (Janssen Pharmaceutica) 5-HT_2 receptor antagonists ketanserin and pirenpirone (see Figure 6-4, to follow, for chemical structures of ketanserin and pirenpirone relative to AMI-193). Figure 6-2 shows the potent dose-related antagonism of the DOM stimulus by pirenpirone (AD_{50} = 0.012 mg/kg), and antagonism of DOM-stimulus generalization to the hallucinogen (+)LSD and 5-OMe DMT (AD_{50} = 0.01 and 0.02 mg/kg, respectively) [18]. Likewise, using rats trained to discriminate (+)LSD from vehicle, the (+)LSD stimulus was potently antagonized by pirenpirone [19]. In fact, Colpaert et al., [19] referred to pirenpirone as a "specific" hallucinogen (i.e., LSD) antagonist rather than

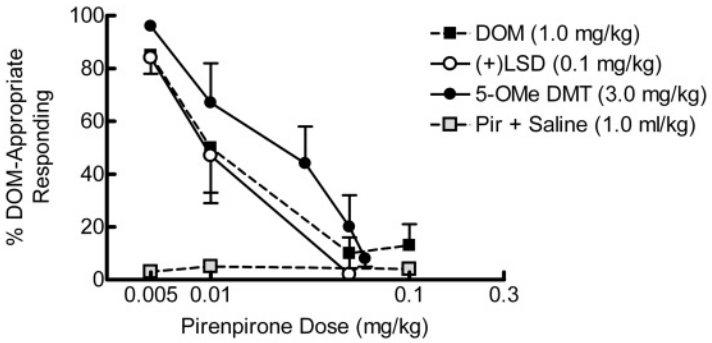


Figure 6-2. Dose-related antagonism of the DOM stimulus by the 5-HT_2 receptor antagonist pirenpirone (Pir) (administered 45 minutes prior to testing), and of DOM-stimulus generalization to (+)LSD and 5-OMe DMT, in rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle. Administration of the 5-HT_2 receptor antagonist pirenpirone in combination with saline resulted in saline-appropriate responding. Saline produced <20% DOM-appropriate responding.

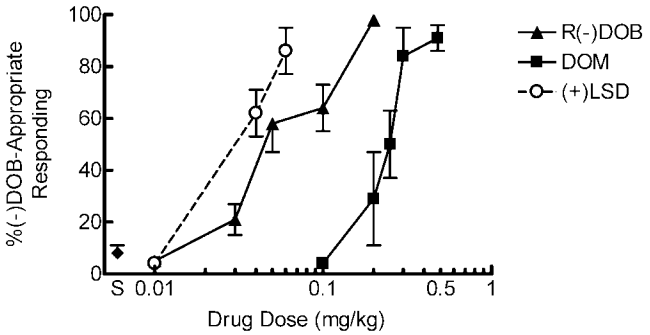


Figure 6-3. Results of stimulus generalization studies in rats (n = 4–5) trained to discriminate 0.2 mg/kg of R(-)DOB from saline vehicle. A 15-minute pre-session injection interval and intraperitoneal route of administration was used for all agents. S = saline (1.0 ml/kg).

as a 5-HT_2 receptor antagonist. 5-HT_2 receptor antagonists are now widely recognized to block the discriminative stimulus effects of *classical hallucinogens*.

DOM-stimulus generalization occurred to the potent classical hallucinogen DOB and its *R*(−)-isomer (ED_{50} = 0.2 and 0.1 mg/kg, respectively), Both agents were later determined to be 5-HT_2 receptor agonists, and *R*(−)DOB was employed as a training drug in rats [20, 21]. The *R*(−)DOB stimulus (training dose = 0.2 mg/kg) (ED_{50} = 0.05 mg/kg) generalized to (+)LSD (ED_{50} = 0.04 mg/kg), DOM (ED_{50} = 0.24 mg/kg) (Figure 6-3), and *S*(+)DOB (ED_{50} = 0.56 mg/kg). Here too, pirenpirone potently antagonized the stimulus effects of 0.2 mg/kg of *R*(−)DOB in a dose-related manner (AD_{50} = 0.03 mg/kg) [21].

DOI, the iodo counterpart of DOB, also served as a training drug in rats at a dose of 0.5 mg/kg [22]. The DOI stimulus generalized to *R*(−)DOI (ED_{50} = 0.16 mg/kg), *S*(+) DOI (ED_{50} = 0.34 mg/kg), DOM (ED_{50} = 0.49 mg/kg), (+)LSD (ED_{50} = 0.05 mg/kg), and was antagonized by pretreatment of the animals with the 5-HT_2 receptor antagonist ketanserin [22].

Once again, the case was closed (or, apparently, seemingly so). The "great mystery" had been solved for a second time. Classical hallucinogens act as 5-HT_2 receptor agonists.

3. Multiple Types of 5-HT_2 Receptors

As things would have it, 5-HT_2 receptors were eventually found to represent a "family" of receptors consisting of 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor subtypes [3]. So the next question became: "which one (or more) of the three 5-HT_2 receptor subtypes is involved in the mechanism of action of classical hallucinogens as discriminative stimuli?" Classical hallucinogens showed little selectivity for these three 5-HT_2 receptor subtypes and displayed a nearly equivalent binding affinity for each [23]. What was required to sort through this problem was a 5-$HT_{2A,}$ 5-HT_{2B}, or 5-HT_{2C}-selective antagonist. The very first 5-HT_{2A}-selective antagonist effective in behavioral studies of this

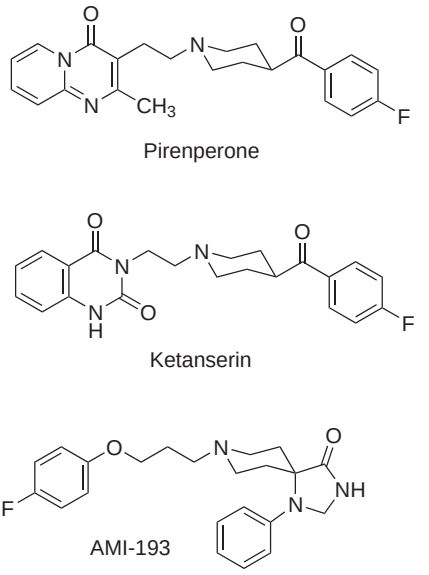


Figure 6-4. Chemical structures of the 5-HT_2 receptor antagonists ketanserin, pirenpirone, and AMI-193.

type was AMI-193. AMI-193 (Figure 6-4) showed >1,000-fold selectivity for 5-HT_{2A} *versus* 5-HT_{2C} receptors and potently blocked the discriminative stimulus effects of DOM [24]. It was concluded that the DOM stimulus functions through a 5-HT_{2A} receptor-mediated agonist mechanism. Fiorella et al. [25] and Schreiber et al. [26] subsequently confirmed that classical hallucinogens produce their discriminative stimulus effects through a 5-HT_{2A} rather than 5-HT_{2C} receptor mechanism.

Finally, the story had come full circle. Classical hallucinogens (i.e., phenylalkylamines, such as DOM and related compounds) and indolealkylamines such as LSD—although indolealkylamines are fairly nonselective with regard to interaction at different serotonin receptor subtypes—probably produce their *common* discriminative stimulus effects *via* activation of 5-HT_{2A} serotonin receptors. It might be noted that the early studies using peripheral rat fundus serotonin receptors had been vindicated. That is, serotonin receptors present in rat fundus have since been shown to represent 5-HT_{2B} serotonin receptors, and the various phenylalkylamine hallucinogens (i.e., DOM derivatives) show almost no selectivity for binding to 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors [23]. It might also be noted that AMI-193, the 5-HT_{2A} versus 5-HT_{2C}-selective antagonist mentioned above, does not bind at 5- HT_{2B} receptors.

Classical hallucinogens seem to act as 5-HT_{2A} serotonin receptor agonists, the DOM stimulus was shown to be antagonized by two "5-HT_2-selective" antagonists (ketanserin and pirenpirone), and was later demonstrated to be antagonized by the "5-HT_{2A}-selective antagonist" AMI-193. If classical hallucinogens act *via* a 5-HT_{2A} receptor agonist mechanism, it might be possible to demonstrate a relationship between stimulus generalization potency, using DOM as training drug, and the affinities of these

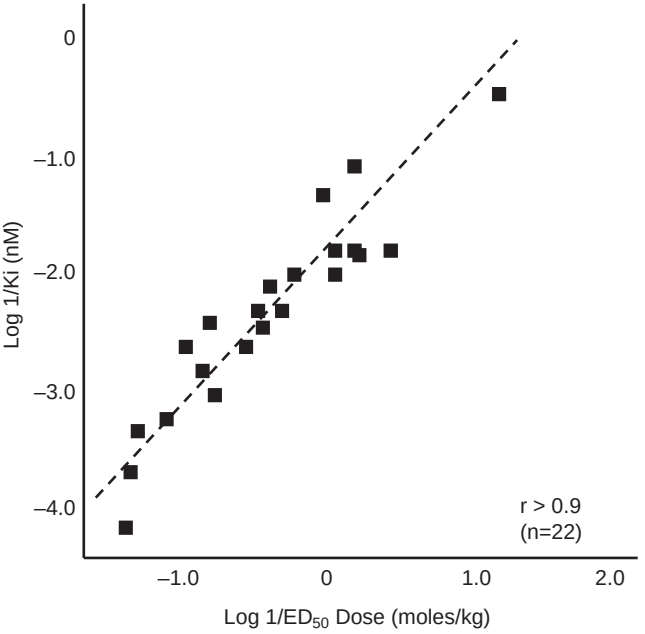


Figure 6-5. Relationship between the stimulus generalization potencies of a series of classical hallucinogens and their affinities for 5-HT_{2A} receptors (n = 22, r > 0.9) [17].

agents for 5-HT_{2A} receptors. Indeed, for 22 agents, stimulus generalization potency was highly correlated with 5-HT_{2A} receptor affinity (Figure 6-5) [17].

As might have been expected, as mentioned above, agents acting *via* a specific putative mechanism were shown to be antagonized by antagonists for that mechanism. For example, the stimulus effects of amphetamine—an agent thought to be indirectly mediated via a dopaminergic mechanism—were antagonized by dopamine receptor antagonists, and opioids—thought to act *via* an opioid receptor mechanism—were antagonized by opioid antagonists. And, there are other examples. But, this was the first time that the discriminative stimulus effects of a class of agents, whose mechanism of action was previously unknown, was shown to be mediated by a specific receptor mechanism using drug discrimination techniques. And, this was accomplished by meeting several different tests (*vide supra*) deemed to be of consequence: 1) stimulus generalization to a known serotonergic agent (i.e., the 5-HT releasing agent fenfluramine—implicating a general serotonin (i.e., 5-HT) receptor mechanism); 2) antagonism by the non-(5-$HT_{2A\ vs.\ 2C}$) selective 5-HT_2 receptor antagonists ketanserin and pirenpirone—implicating a direct 5-HT_2 receptor mechanism); 3) antagonism by a "5-HT_{2A}-selective" receptor antagonist, AMI-193—implicating a 5-HT_{2A} receptor mechanism); and 4) a correlation between stimulus generalization potency and 5-HT_{2A} receptor affinity (indicating that the effects are directly receptor-mediated as opposed to being mediated by 5-HT release, reuptake, allosteric, or some other serotonergic-related mechanism). Clearly, a 5-HT_{2A} receptor agonist mechanism had been implicated

as the underlying basis for the actions of the phenylalkylamine and indolealkylamine "classical hallucinogens."

Finally, as described in Chapter 5, both the stimulus generalization potencies of a series of classical hallucinogens (using DOM-trained animals), and the 5-HT_{2A} receptor affinities of these same agents, were shown to be significantly correlated with their human hallucinogenic potencies. A *specific mechanistic* link had been established; classical hallucinogens act as agonists at 5-HT_{2A} serotonin receptors. Hence, the stimulus actions of a group of agents, the "*classical hallucinogens*," whose mechanism of action had been previously unknown, were related to a specific neurotransmitter receptor mechanism: activation of 5-HT_2, more specifically, 5-HT_{2A}, serotonin receptors. Indeed, this was the first instance where the stimulus effects of a group of agents whose mechanism of action was directly linked to a specific receptor-mediated mechanism using drug discrimination techniques. Although classical hallucinogens produce effects in humans that differ in their subtlety, their activation of 5-HT_{2A} receptors seems to be the one feature they all have in common (i.e., referred to as the "*common component hypothesis*") [2]. Some of these classical hallucinogens, notably indolealkylamine or tryptamine hallucinogens, are quite promiscuous and appear to activate several different populations of 5-HT receptors; others, particularly α-alkyltryptamines, behave as inhibitors of monoamine oxidase. But, regardless of whatever else they might do, they appear subservient to a 5-HT_{2A} serotonin receptor agonist mechanism.

C. AMPHETAMINE-RELATED STIMULANTS

1. Amphetamine, Dopamine, and Norepinephrine

One of the most readily recognized members of the psychostimulant family is the phenylisopropylamine analog amphetamine, and amphetamine is believed to produce many (if not most) of its behavioral effects via a neurotransmitter transporter mechanism involving release of the neurotransmitters NE and DA [27]. That is, amphetamine is thought to act as an indirect receptor agonist by releasing certain neurotransmitters (primarily NE and DA). Amphetamine is a nonselective NE, DA, and 5-HT releasing agent that also inhibits the reuptake of each of these neurotransmitters [28]. With respect to both actions (i.e., neurotransmitter release and neurotransmitter reuptake), the effect of amphetamine is more pronounced on NE and DA than on 5-HT and, in this regard, amphetamine is more potent on the release than the reuptake mechanism [28]. In fact, (+)amphetamine is 3-fold more potent in releasing NE than DA [27]. Over the years, many drug discrimination studies based on amphetamine-stimulus generalization to a variety of dopamine receptor agonists, administration of catecholamine neurotransmitter precursors, and electrical stimulation of dopaminergic neurons in the brain, together with stimulus antagonism studies utilizing various dopamine receptor antagonists, provided defining evidence for dopaminergic involvement in the discriminative stimulus effects of (+)amphetamine [reviewed: 29, 30]. In contrast, there is little evidence of a role for 5-HT (e.g., using serotonin receptor agonists, serotonin releasing agents such as fenfluramine—see Figure 6-6 for chemical structure—serotonin receptor pre-

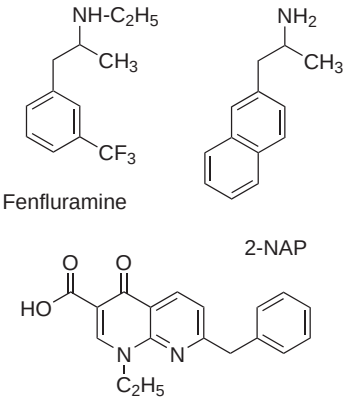


Figure 6-6. Chemical structures of fenfluramine, 2-NAP, and amfonelic acid (AFA).

cursors, or various 5-HT receptor antagonists) [29,30]. However, evidence exists, controversial though it might be, for a possible role for a noradrenergic mechanism in the stimulus actions of (+)amphetamine. Involvement of the latter has not been extensively studied, but (+)amphetamine-trained animals recognized the NE reuptake inhibitor nisoxetine, and, furthermore, nisoxetine-trained animals recognized (+)amphetamine. This was first reported by Snoddy and Tessel [e.g., 31, and see 29 and 30 for additional discussion]. This could be a species-related, dose-related, and/or methodological phenomenon; but, this still needs to be sorted out. To date, there seems to be a very limited role for 5-HT in the stimulus actions of (+)amphetamine (but, see below); a possible role for NE (this desperately requires further investigation), and an almost certain role for DA has been established.

Other agents that possess a neurotransmitter release profile similar to that of amphetamine (i.e., DA and NE > 5-HT) include methamphetamine, cathinone, and methcathinone [27, 32], and each of these agents substituted in (+)amphetamine-trained animals as described in previous chapters. That is, higher potency for the release (or inhibition of reuptake) of DA and/or NE than for 5-HT favors amphetamine-like discriminative stimulus effects.

Supportive of dopaminergic involvement in the stimulus actions of (+)amphetamine is that the selective DA reuptake inhibitor amfonelic acid (see Figure 6-6 for chemical structure) fully substituted for (+)amphetamine in (+)amphetamine-trained animals (Figure 6-7) [33]. Indeed, using rats trained to discriminate amfonelic acid, the amfonelic acid stimulus generalized to (+)amphetamine, cathinone, and cocaine [34]. In contrast, rats depleted of dopamine learned and maintained the (+)amphetamine stimulus as efficiently as control animals [35]. So, perhaps, there is more to the (+) amphetamine stimulus than simple (direct or indirect) activation of dopaminergic mechanisms.

A group of agents structurally related to the phenylisopropylamines amphetamine and methamphetamine (i.e., more specifically, reduced analogs of cathinone and

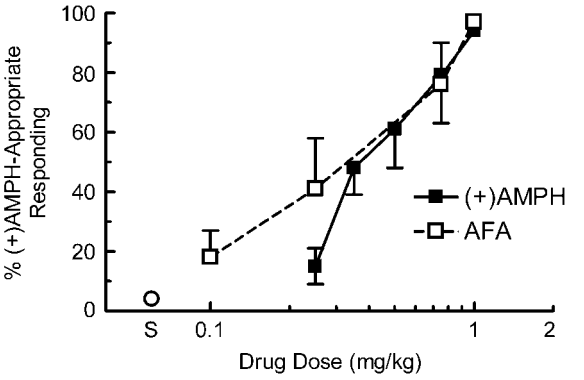
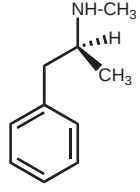
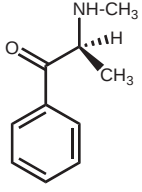
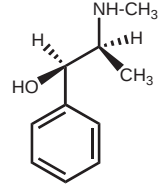
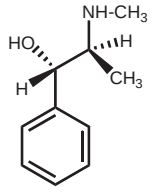
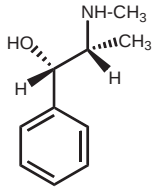
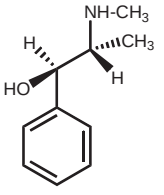


Figure 6-7. Results of stimulus generalization studies with the dopamine reuptake inhibitor amfonelic acid (AFA) in rats trained to discriminate 1.0 mg/kg of (+)amphetamine from vehicle [33]. S = effect of saline.

methcathinone) are the phenylpropanolamines (see Figure 6-8). Two of these phenylpropanolamines, (−)ephedrine and (+)norpseudoephedrine, substituted in (+)amphetamine-trained animals (Table 4-1). As discussed in Chapter 4, the majority of the isomers substituted in animals trained to discriminate (−)ephedrine from vehicle [36]. The ability of these compounds to act as neurotransmitter releasing agents and neurotransmitter reuptake inhibitors was examined. There was a statistically significant correlation between the potencies of these agents to release norepinephrine (NE) and their stimulus generalization potencies in (−)ephedrine-trained animals [32]. It was concluded, on the basis of this and other studies, that (−)ephedrine produces it discriminative stimulus effects primarily via release of NE. There was also a correlation between the potencies of these same compounds to release NE and their potencies to release DA even though they were less potent DA releasing agents than NE releasing agents [32]. Interestingly, the two most potent dopamine releasers were (−)ephedrine and (+)norpseudoephedrine—the only two phenylpropanolamines that substituted in (+)amphetamine-trained animals. In general, the phenylpropanolamines were inactive at releasing 5-HT [32].

Increasing the serotonergic nature of an agent, even when it can act upon all three neurotransmitter transporters (i.e., NET, DAT, SERT), seems to detract from its ability (or potency) to substitute for a (+)amphetamine stimulus. For example, benz-fusion at the *c-face* of amphetamine affords the amphetamine analog 2-NAP (see Figure 6-6 for chemical structure) which failed to substitute in (+)amphetamine-trained rats at doses of up to nearly ten times the ED_{50} dose of (+)amphetamine (i.e., 2-NAP produced 14% amphetamine-appropriate responding at the highest dose evaluated) [37]. It is now recognized that although 2-NAP is approximately as potent as (+)amphetamine and (+)methamphetamine in releasing DA and NE (EC_{50} *ca*. 5–15 nM), it is substantially more potent (EC_{50} = 3.4 nM) than (+)amphetamine (EC_{50} = 1,756 nM) at releasing 5-HT [27, 38, 39]. Thus, release of 5-HT might interfere with amphetamine-like discriminative stimulus actions. This finding is consistent with a proposal by Rothman and co-workers

Phenylisopropylamines	Phenylpropanonamines	Phenylpropanolamines			
					
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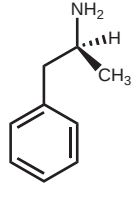
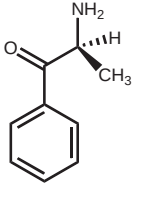
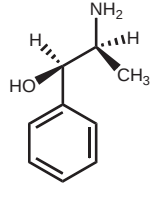
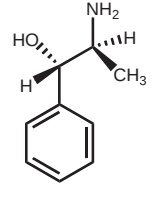
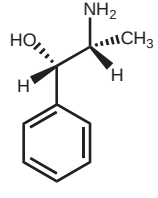
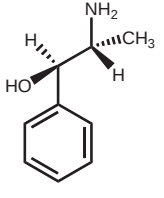
					
S(+)Amphetamine	S(-)Cathinone	(-)Norephedrine	(+)Norpseudo-ephedrine	(+)Norephedrine	(–)Norpseudo-ephedrine

Figure 6-8. General chemical structures of the phenyisopropylamines amphetamine and methamphetamine, the phenylpropanonamines cathinone and methcathinone, and the reduced phenylpropanonamines (i.e., phenylpropanolamines). See text and Chapter 4 for further discussion about stereochemistry.

who have suggested that a balance must exist between the releasing effects of DA and 5-HT in order for agents to produce their overt effects, and that increased 5-HT release attenuates the stimulant (e.g., locomotor stimulation and self-administration) effects mediated by DA release [39, 40]. The same might be true with the discriminative stimulus actions of these agents (*vide supra*).

All three monomethoxy positional isomers of amphetamine, PMA, MMA, and OMA, produced amphetamine-like stimulus effects with a potency of (+)amphetamine > (−)amphetamine > PMA > MMA > OMA (see Chapter 4 for discussion and Figure 4-2 for the chemical structures of these agents). Based on neurotransmitter release and reuptake studies, this order of potency can only be explained by the ability of these agents to release, or inhibit the reuptake of, NE and/or DA as described by Tseng and co-workers [28]. Had 5-HT release, or inhibition of 5-HT reuptake, been their primary mechanism of action, PMA and MMA might have been expected to be more potent than (+)amphetamine. Furthermore, the 8-fold reduced potency of PMA (ED_{50} = 1.9 mg/kg) relative to (+)amphetamine (ED_{50} = 0.44 mg/kg) for substitution in (+)amphetamine-trained animals (see Chapter 4) might be related to the enhanced ability of PMA over (+)amphetamine to release 5-HT.

Although it remains to be fully investigated, another case in question involves the TAP positional isomers (see Chapter 4 and Figure 4-1). *p*TAP and *m*TAP are similar in potency as releasers of DA and NE (EC_{50} = 18 to 44 nM) [39]. However, both agents are more potent at releasing 5-HT than (+)amphetamine [39]. Neither agent substituted for (+)amphetamine (*vide supra*). Perhaps, although this remains to be determined, *o*TAP will display reduced potency for 5-HT release (or reuptake) than its positional isomers because, of the three TAP positional isomers examined, only *o*TAP substituted in (+)amphetamine-trained rats. Additional studies are warranted. It might also be noted that (+)methamphetamine, a more potent positional isomer of *o*TAP (see Chapter 4/section A), possesses a neurotransmitter release profile similar to that of (+)amphetamine [41].

Cocaine substitutes for a (+)amphetamine stimulus and *vise versa* [30]. Here is another classical *exampl*e of a "*general*," but "*non-specifi*c," mechanism in the actions of two agents (see also: benzodiazepines and barbiturates as discussed in Chapter 3). Cocaine behaves primarily as a NE/DA/5-HT reuptake inhibitor and is nearly equipotent in this regard [41]. Amphetamine is primarily a NE/DA releasing agent. Yet, both compounds produce similar stimulus effects in animals. Perhaps it is the action of cocaine at the 5-HT transporter that contributes to its reduced potency relative to (+)amphetamine in tests of stimulus generalization.

To date, the results of transporter studies and drug discrimination studies are relatively consistent: 1) DA release, or blockade of DA reuptake, supports involvement of a dopaminergic mechanism in the stimulus actions of (+)amphetamine, and the actions of amphetamine-related psychostimulants; 2) the (+)amphetamine stimulus is mediated by a DA and/or NE mechanism; and 3) the (+)amphetamine stimulus does not seem to involve (but see below) a 5-HT transporter-related mechanism, and agents with enhanced serotonergic action, as seen with indirect 5-HT receptor agonists, detract from, or abolish, amphetamine-like stimulus actions even if they are capable of releasing or blocking the reuptake of DA/NE.

2. Amphetamine and Serotonin

There is conflicting evidence that the neurotransmitter 5-HT modulates the stimulus effects of (+)amphetamine [29]; however, further studies are required with serotonergic agents. For example, the 5-HT_{1A} receptor agonist 8-OH DPAT has been demonstrated to attenuate the stimulus effects of (+)amphetamine in monkeys [42]. Because Pzegalinski and Filip [43] found that 8-OH DPAT had no effect in Wistar rats trained to discriminate (+)amphetamine from saline vehicle, they suggested this might be a species-related phenomenon. Indeed, the latter findings were later substantiated using male Sprague-Dawley rats; that is, 8-OH DPAT neither substituted for, nor antagonized, a (+)amphetamine stimulus [44]. Interestingly, however, low doses (i.e., ≤0.1 mg/kg) of 8-OH DPAT potentiated the effect of the ED_{50} dose of (+)amphetamine such that, when administered in combination, the ED_{50} dose of (+)amphetamine plus 0.1 mg/kg of 8-OH DPAT resulted in (+)amphetamine-stimulus generalization (i.e., >80% drug-appropriate responding) [44]. Moreover, pretreatment of (+)amphetamine-trained rats with very low (i.e., 0.01 and 0.1 mg/kg) doses of 8-OH DPAT produced a leftward shift of the (+)amphetamine dose-response curve [44]. Clearly, 8-OH DPAT influences (+) amphetamine-appropriate responding under the conditions evaluated (see also Chapter 3). (+)Methamphetamine and (+)amphetamine substitute for one another regardless of which agent was used as training drug in rats (*vide supra*). In methamphetamine-trained rats, 8-OH DPAT neither substituted for, nor antagonized, the methamphetamine stimulus [45]. This is consistent with what has been described in studies with (+)amphetamine in rats. However, in pigeons trained to discriminate methamphetamine from saline vehicle, 8-OH DPAT substituted for methamphetamine at high doses, but antagonized the methamphetamine stimulus at low 8-OH DPAT doses [46]. The exact mechanistic involvement of 5-HT_{1A} serotonin receptors in the stimulus actions of amphetamine and methamphetamine remains to be elucidated, but there is certainly evidence that this receptor population might play a modulatory role. In fact, a review of the literature provides some tantalizing information. Chen and Reith [47] demonstrated that stimulation of 5-HT_{1A} receptors by administration of low doses of 8-OH DPAT might act at pre-synaptic 5-HT_{1A} receptors to modulate dopamine (and 5-HT) release, and act at postsynaptic 5-HT_{1A} receptors to modulate NE release and also activate dopamine D_2 receptors. Also, Done and Sharp found that 8-OH DPAT increases the efflux of NE in rat hippocampus microdialysis studies [48].

Likewise, although 5-HT_3 serotonin receptor antagonists failed to attenuate the stimulus effects of (+)amphetamine [e.g., 49, 50], pretreatment of (+)amphetamine-trained rats with the 5-HT_3 receptor partial agonist MD-354 (*meta*-chlororphenylguanidine; Figure 6-9) potentiated the stimulus effects of low (+)amphetamine doses [51]. Recent studies suggest that MD-354 might exert some of its actions *via* an α_2-adrenoceptor mechanism [52]; this offers a possible explanation that requires further investigation. Also, the 5-HT_6 receptor antagonist MS-245 (i.e., 1-benzenesulfonyl-5-methoxy-*N*,*N*-dimethyltryptamine; Figure 6-9) potentiated the stimulus effects of (+)amphetamine [53]. Although MS-245 is a fairly selective 5-HT_6 receptor antagonist, it binds with only 10-fold selectivity for 5-HT_6 *versus* DA receptors [53]; it is unknown whether this agent is a DA receptor agonist or antagonist. There is also evidence that interaction of

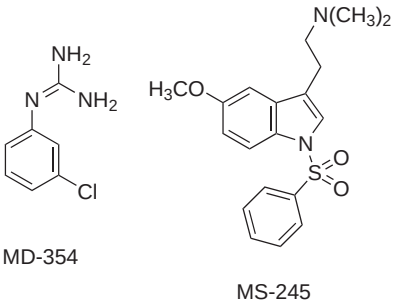


Figure 6-9. Chemical structures of the 5-HT_3 receptor partial agonist MD-354 and the 5-HT_6 receptor antagonist MS-245.

agents with 5-HT_6 receptors can modulate the effects of dopamine at dopamine- and cAMP-regulated phosphoproteins (e.g. DARPP-32) [54]. For further discussion, see, Young et al., [55]. This provides a potential explanation for the action of MS-245 on the (+)amphetamine stimulus. Thus, the actions of certain "*selective*" serotonergic agents might modulate the stimulus effects of amphetamine by their indirect action on DA or NE mechanisms.

Taken together, the literature suggests that amphetamine seems to produce its discriminative stimulus effects via either an indirect DA and/or NE receptor mechanism. But, its stimulus effects appear to be modulated (antagonized or potentiated) by 5-HT receptor agents. Further studies are warranted.

D. MDA AND MDMA

1. MDA as a Discriminative Stimulus

One of the most interesting agents to be investigated in drug discrimination studies is, ostensibly, the phenylisopropylamine MDA or 1-(3,4-methylenedioxyphenyl)-2-aminopropane (see Figure 6-10 for chemical structure). This substance was available on the illicit market during the 1960s (i.e., as the "*Love Drug*") and reappeared in the 1990s and more recently as a so-called "designer drug." Anecdotal reports during the 1960s suggested that ingestion of MDA produces effects similar to a combination of the central stimulant *cocaine*, and the hallucinogenic agent *LSD*. Interestingly, in tests o*f stimulus generalization, MDA was the very first agent shown to substitute* both in animals trained to discriminate the phenylisopropylamine central stimulant (+)amphetamine and the phenylisopropylamine classical hallucinogen DOM from saline vehicle (ED_{50} = 2.29 and 1.68 mg/kg, respectively) [56] (see Figure 4-22). Although MDA was not as potent as either of the training drugs, it was unique in that it produced both types of stimulus effects. MDA is an optically active agent that exists as a pair of optical isomers: *S*(+)MDA and *R*(−)MDA. Marquardt et al. [57] examined the general pharmacological actions of both MDA optical isomers and noted differences: *R*(−)MDA behaved more like the hallucinogen LSD whereas *S*(+)MDA behaved more like the

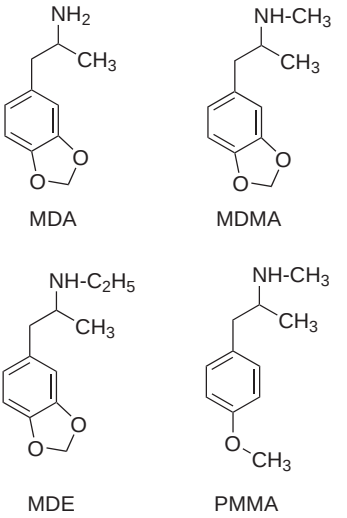


Figure 6-10. Chemical structures of MDA, MDMA, MDE, and PMMA showing their structural relationship.

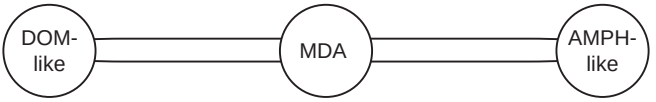


Figure 6-11. An initial concept suggesting that certain phenylisopropylamines exist on a DOM-like to (+)amphetamine-like stimulus continuum with MDA, because it produced both types of effects, existing somewhere in between [adapted from 59].

psychostimulant amphetamine. A subsequent examination of the individual isomers in tests of stimulus generalization revealed that *S*(+)MDA (ED_{50} = 0.90 mg/kg), but not *R*(−)MDA substituted in rats trained to discriminate (+)amphetamine from vehicle, and that *R*(−)MDA (ED_{50} = 0.81 mg/kg), but not *S*(+)MDA substituted in rats trained to discriminate DOM from vehicle [58] (see Figure 4-22). Clearly, the two optical isomers of MDA produce distinct (i.e., stereospecific) stimulus effects. Furthermore, where stimulus generalization occurred, the MDA isomers were approximately twice as potent as their racemate in the two respective groups of animals.

Initial thinking was that the stimulus effects of phenylisopropylamines might exist on a DOM-like to amphetamine-like continuum (Figure 6-11) and that MDA, because it produced both effects, resides somewhere near the middle of the continuum.

Rats were later trained to discriminate MDA from vehicle and, consistent with the above findings, the MDA stimulus generalized to the psychostimulants (+)amphetamine (ED_{50} = 1.93 mg/kg) and cocaine (ED_{50} = 5.9 mg/kg) and to the hallucinogens DOM (ED_{50} = 0.61) (Figure 6-12) and (+)LSD (data not shown; ED_{50} = 0.058 mg/kg) [60, 61]. MDA stimulus generalization also occurred to both MDA isomers with *S*(+)MDA

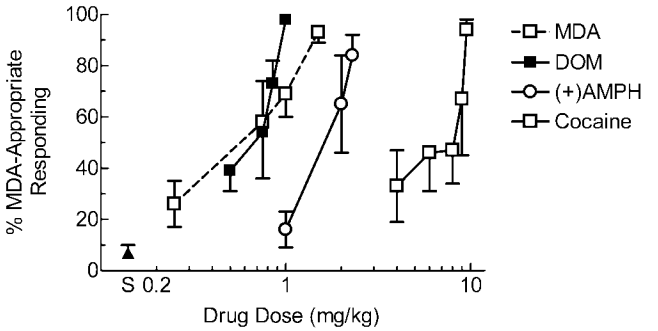


Figure 6-12. Results of stimulus generalization studies using rats trained to discriminate 1.0 mg/kg of MDA from saline vehicle. S = effect of saline. See text for discussion.

(ED_{50} = 0.51 mg/kg) being similar in potency to (±)MDA (ED_{50} = 0.65 mg/kg) and about twice as potent as *R*(−)MDA (ED_{50} = 1.18 mg/kg). From this perspective, MDA was quite unique among the phenylisopropylamines in terms of its hallucinogenic / stimulant classification.

If the optical isomers of MDA produce distinct stimulus effects, it should be possible to train animals to discriminate *S*(+)MDA from *R*(−)MDA from vehicle in a three-lever operant paradigm. That is, if two agents, or for that matter two optical isomers (as in the present instance), produce distinct or unique stimulus effects, it should be possible to train animals to recognize these differences. Indeed, it was possible to train rats to discriminate 1.25 mg/kg of *S*(+)MDA, from 1.25 mg/kg of *R*(−) MDA, from saline vehicle [62]. Clearly, the two optical isomers of MDA produced distinct stimulus effects! Administration of psychostimulants such as (+)amphetamine and cocaine (which—recall: act by different mechanisms) elicited *S*(+)MDA-appropriate responding, and administration of hallucinogens such as the phenylisopropylamine hallucinogen DOM, the indolealkylamine hallucinogen (+)LSD, and the phenylethylamine hallucinogen mescaline elicited *R*(−)MDA-appropriate responding, in a dose-dependent fashion. And, as might have been expected on the basis of mechanistic discussions in Chapter 6, section A, and the binding profile of the MDA optical isomers [63], the 5-HT_2 antagonist pirenpirone potently antagonized the stimulus effects of *R*(−) MDA but was without effect on the stimulus actions of *S*(+)MDA [62].

2. MDMA as a Novel Stimulus

In the 1980s a novel substance appeared on the open market, and later (after it was Scheduled) on the clandestine market: the designer drug *N*-methyl-1-(3,4-methylenedioxyphenyl)-2-aminopropane (known as MDMA, XTC, and by a variety of other names). MDMA is simply the *N*-monomethyl analog of MDA. Structure-activity studies on MDMA using drug discrimination techniques were conducted in two different laboratories [for a brief overview, see: 59, 64]. On the basis of established phenylisopropylamine SAR (see Chapter 5), *N*-monomethylation of MDA should enhance its

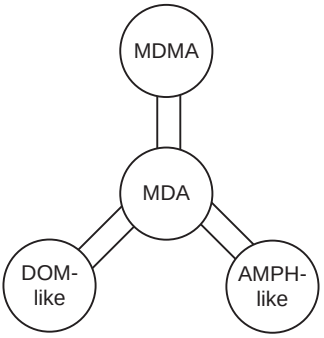


Figure 6-13. A trifurcated model to explain the discriminative stimulus effects of phenylisopropylamines [adapted from 59].

amphetamine-like stimulus properties and diminish (or abolish) its DOM-like stimulus action. It became apparent, using animals trained to discriminate MDMA from vehicle, that the classification scheme shown as Figure 6-11 was too simplistic to explain the findings. That is, certain agents substituted in MDMA-trained animals but did not substitute in either DOM- or (+)amphetamine-trained animals. For example, MDE, the *N*-ethyl homolog of MDMA failed to substitute in rats trained to discriminate either DOM- or (+)amphetamine from vehicle but substituted in animals trained to discriminate MDMA [58]. To account for these, and other, findings, a new model was proposed to explain the stimulus effects produced by phenylisopropylamines (Figure 6-13).

This new model (Figure 6-13) was short-lived because it failed to explain certain key findings: for example, that MDMA stimulus generalization occurred to (+)amphetamine, suggesting that MDMA possesses some amphetaminergic character. Furthermore, a new designer drug, PMMA (see Figure 6-10 for chemical structure), the *N*-monomethyl analog of PMA, substituted in MDMA-trained animals, but not in (+)amphetamine-trained (or DOM-trained) animals, suggesting that it lacked amphetamine-like (or DOM-like) action [65, 66].

In fact, PMMA was several times more potent than MDMA in tests of stimulus generalization regardless of which of the two was used as training drug [67]. It was further suggested that PMMA might represent the structural parent of MDMA because the two structures differed only with respect to the presence of an oxygen atom (see Figure 6-10). It should be noted, however, that the stimulus effects produced by PMMA and MDMA are not identical; for example, the PMMA stimulus, unlike an MDMA stimulus, failed to generalize completely to the psychostimulant cocaine or to *R*(–) MMA [68].

3. The Venn Model

Using rats trained to discriminate 1.25 mg/kg of PMMA from vehicle, stimulus generalization occurred to *S*(+)PMMA (ED_{50} = 0.41 mg/kg) whereas administration of *R*(–) PMMA elicited a maximum of only 62% PMMA-appropriate responding [69]. An

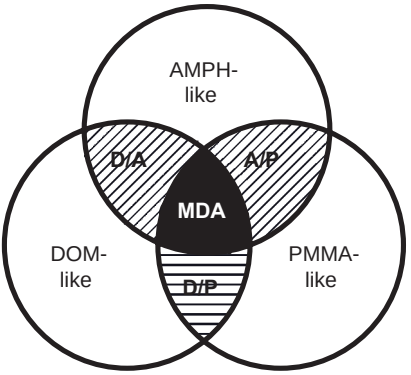


Figure 6-14. A Venn diagram illustrating the relationships between various phenylalkylamines of abuse based on their discriminative stimulus properties (adapted from 67).

interesting finding was that the PMMA stimulus generalized to both isomers of the dimethoxyphenylisopropylamine 3,4-DMA (see Figure 5-3 for chemical structure) (ED_{50} = 2.6 and 3.9 mg/kg for the *S*(+)- and *R*(−) isomers, respectively)—an agent to which neither a DOM nor (+)amphetamine stimulus generalized [70]. It might be noted that the MDA stimulus also generalized to 3,4-DMA [59] suggesting that MDA might possess an additional component of action unrelated to its amphetamine-like and DOM-like stimulus qualities.

Administration of *S*(+)MDA, *R*(−)MDA and (±)MDA to rats trained to discriminate 1.25 mg/kg of PMMA from saline vehicle resulted in substitution in each case. (±)MDA and *S*(+)MDA were nearly equipotent and several-fold more potent than *R*(−) MDA [71].

A new model (a Venn model) was proposed (Figure 6-14) [67, and see 2 for a review]. This model seems to account for many of the findings to date. That is, rats can be trained to recognize or discriminate (at least) three distinct structure-types of phenylalkylamines: the hallucinogen DOM, the psychostimulant (+)amphetamine, and the designer drug PMMA from saline vehicle. Animals trained to discriminate any one of these three agents did not recognize (i.e., stimulus generalization did not occur upon administration of) the other two. What is implied is that the three individual agents, DOM, (+)amphetamine, and PMMA, possess different structure-activity relationships and, very likely, different mechanisms of action. This model further implies that some phenylalkylamines can produce more than one type of stimulus effect and probably do so by more than one mechanism of action.

For example, racemic MDA was recognized by all three groups of animals: animals trained to discriminate DOM, (+)amphetamine, and PMMA from vehicle. Hence, racemic MDA falls into the common convergence or intersect of all three aspects of the Venn diagram (Figure 6-14). In contrast, *R*(−)MDA produced only DOM- and PMMA-like effects, suggesting it is a D/P-type agent (see Figure 6-14), whereas *S*(+) MDA substituted only in (+)amphetamine- and PMMA-trained animals (suggesting it is an A/P-type agent).

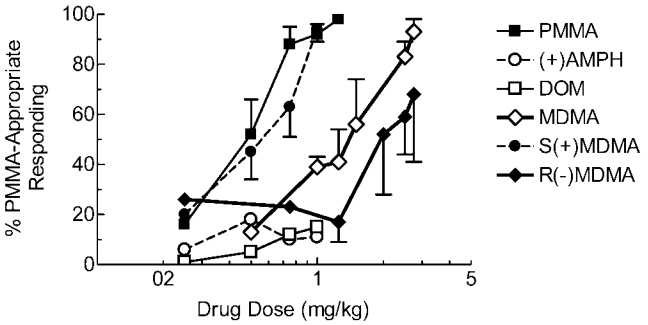


Figure 6-15. Results of stimulus generalization studies employing PMMA (1.25 mg/kg) as training drug. Saline produced 3% drug-appropriate responding (data not shown).

MDMA produced PMMA-like stimulus effects but also displays amphetaminergic character (as noted in drug discrimination and other studies) suggesting it is best classified as an A/P-type agent. However, PMMA-like responding was attributable to *S*(+)MDMA with *R*(−)MDMA producing a maximum of only 68% PMMA-appropriate responding followed by disruption of behavior upon administration of higher drug doses (Figure 6-15) [67].

MBDB, the α-ethyl homolog of MDMA, is an MDMA-like agent that lacks amphetamine-like stimulus properties, but MBDB and both its optical isomers substituted in animals trained to discriminate MDMA from vehicle [64]. Consistent with these findings, and with the model shown in Figure 6-14, the PMMA stimulus generalized to *S*(+)MBDB (ED_{50} = 0.8 mg/kg) and *R*(−)MBDB (ED_{50} = 2.0 mg/kg) [70].

The results from drug discrimination (i.e., stimulus generalization) studies are not only consistent with the proposed model but also identify (±)MDA as the first phenylalkylamine shown to produce all three types of stimulus effects (i.e., MDA produces amphetamine-like, DOM-like, and PMMA-like effects) in rats [71]. With the exception of the *D*/A intersect, for which no agent has yet been identified, various other agents have been identified that fall into the various categories and intersects [2, 70]. Not all DMA and TMA analogs (see Figure 5-3) have yet been investigated. But certain psychoactive agents whose actions (and mechanism of action) have been hitherto undefined and/or unclassified (e.g., 3,4-DMA, as described above) can now be classified as PMMA-like agents, and 2,4-DMA, which substituted in DOM- and PMMA-trained animals, but not (+)amphetamine-trained animals, can now be classified as *D*/*P* type agent.

With regard to mechanism of action, it would seem that the stimulus effects of DOM are related to activation of 5-HT_2 serotonin receptors and that those of (+)amphetamine involve dopamine (and, perhaps, norepinephrine). The mechanistic underpinnings of PMMA as a discriminative stimulus are still under investigation.

The mechanisms responsible for the stimulus effects of MDMA are somewhat more complex because it is an A/P-type agent. Using MDMA-trained rats, low doses of the 5-HT_{1A} receptor antagonist NAN-190, the 5-HT_2 receptor antagonist pirenpirone,

and the dopamine antagonist haloperidol were able to partially attenuate the MDMA stimulus, but none of these agents decreased MDMA-appropriate responding to less than 46%. However, the 5-HT_3 receptor antagonists zacopride and LY 278584 (AD_{50} = 0.02 µg/kg) antagonized the MDMA discriminative stimulus [49]. Consistent with possible involvement of a 5-HT_{1A} receptor mechanism, MDMA-stimulus generalization occurred to the 5-HT_{1A} receptor agonists (±)8-OH DPAT (ED_{50} = 0.3 mg/kg), *R*(+)8-OH DPAT (ED_{50} = 0.2 mg/kg), and to the 5-HT_{1A} receptor partial agonist *S*(−)8-OH DPAT (ED_{50} = 0.4 mg/kg) [72]. Because MDMA is known to be a 5-HT releasing agent, it was speculated that some of the released 5-HT might activate 5-HT_{1A} (as well as some other 5-HT) receptors [72, 73]. Because MDMA can biochemically increase synaptic levels of dopamine and norepinephrine, in addition to those of 5-HT, and because the released neurotransmitter might conceivably interact with any one of multiple receptor populations, attempts were made to attenuate the MDMA stimulus using several non-selective (i.e., "broad spectrum") receptor antagonists: clozapine, cyproheptadine, and pizotyline (pizotifen; BC-105). Whereas clozapine was without effect, and although cyproheptadine partially antagonized the MDMA stimulus, pizotyline (AD_{50} = 2.5 mg/kg), in combination with the MDMA training dose (1.5 mg/kg) resulted in a dose-dependent decrease in percent drug-appropriate responding to vehicle levels [74]. Despite being able to antagonize the stimulus effects of MDMA by 5-HT_3 receptor antagonists and pizotyline, the exact mechanism of action of MDMA as a discriminative stimulus remains unknown.

E. PMMA

PMMA is the third member, along with DOM and (+)amphetamine, of the triumvirate shown in Figure 6-14. Its mechanism of action as a discriminative stimulus is still unclear. On the basis of established SAR (*vide supra*; Figure 5-8), PMMA should have been a potent psychostimulant. That is, PMMA is the *N*-monomethyl analog of the weak phenylisopropylamine stimulant PMA. However, PMMA lacks any amphetamine-like or central stimulant character [65]. Various 5-HT receptor agonists failed to substitute for, and 5-HT receptor antagonists failed to completely antagonize, the PMMA stimulus (R. Young and R.A. Glennon, unpublished data). Even the nonselective, broad spectrum neurotransmitter antagonists clozapine, cyproheptadine, and pizotifen failed to antagonize the PMMA stimulus [74]. Results suggest, however, that a non-5-HT_2, non-5-HT_{1A} serotonin receptor mechanism might be involved in the stimulus effects of PMMA [68]. PMMA is a 5-HT releasing agent. *S*(+)PMMA is a potent releaser of 5-HT (EC_{50} = 41 nM) and NE (EC_{50} = 147 nM) with reduced activity as a releaser of DA (EC_{50} = 1,000 nM); the *R*(−)isomer of PMMA is a releaser of 5-HT (EC_{50} = 134 nM) with reduced potency for release of NE (EC_{50} = 1,600 nM) and DA (EC_{50} >14,000 nM) (R.B. Rothman, unpublished data). However, *R*(−)PMMA failed to substitute for the PMMA stimulus [69] suggesting that 5-HT release plays a predominant role here, but that additional mechanisms might be involved. Although the PMMA stimulus only partially generalized to the 5-HT/NE releasing agent fenfluramine (EC_{50} values for 5-HT and NE release = 51.7 and 302 nM, respectively) [27], a standard 5-HT releas-

ing agent 4-chloroamphetamine (PCA) fully substituted for the PMMA stimulus (ED_{50} = 0.66 mg/kg) (R. Young and R.A. Glennon, unpublished data). Hence, at least in part, the PMMA stimulus might involve 5-HT release (also see discussion on α-ethyltryptamine in the following section).

The PMMA stimulus is similar to, but distinct from, that produced by MDMA [68]. For example, although the agents substituted for one another regardless of which was used as training drug, MDMA- but not PMMA- stimulus generalization occurred to the psychostimulant cocaine and the 5-HT_{1A} agonist 8-OH DPAT [68], and the MDMA stimulus but not the PMMA stimulus was antagonized by pizotyline [74].

F. α-ETHYLTRYPTAMINE

Phenylethylamines and phenylisopropylamines, collectively referred to as phenylalkylamines, belong to a larger structural class of agents termed arylalkylamines. Arylalkylamines also include indolealkylamines. That is, phenylalkylamines and indolealkylamines both represent classes of arylalkylamines. The indolealkylamine α-ethyltryptamine (α-ET; known clandestinely simply as ET), a homolog of the hallucinogenic agent α-methyltryptamine (see Figure 6-16 for chemical structures), was clinically available as an antidepressant for a short period of time in the early 1960s. In the 1990s it reappeared as a designer drug, and anecdotal reports suggested its effects were similar to those of MDMA. Soon thereafter α-ET was shown to substitute in animals trained to discriminate MDMA from vehicle [75].

α-Ethyltryptamine displays central stimulant and hallucinogenic properties. In tests of stimulus generalization racemic α-ET had been found years earlier to substitute in animals trained to discriminate DOM from saline vehicle [76]. Partial generalization also occurred upon administration of racemic α-ET to (+)amphetamine-trained animals [75]. Because psychoactive phenylalkylamines with abuse potential can produce one or more of three distinct stimulus effects (see Figure 6-14) and because these effects can be stereoselective and even stereospecific, the individual optical isomers of α-ET were synthesized and examined in groups of animals trained to discriminate (+)amphetamine (1.0 mg/kg), DOM (1.0 mg/kg), PMMA (1.25 mg/kg), and MDMA (1.5 mg/kg) from saline vehicle. (–)α-ET (ED_{50} = 7.8 mg/kg), but not (+)α-ET substituted for (+) amphetamine, whereas (+)α-ET (ED_{50} = 2.7 mg/kg), but not (–)α-ET, substituted for

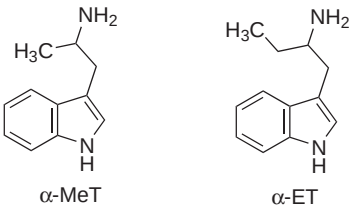


Figure 6-16. Chemical structures of the indolealkylamines α-methyltryptamine (α-MeT) and α-ethyltryptamine (α-ET; ET).

DOM. Both optical isomers of α-ET substituted for PMMA and MDMA with ED_{50} values of 1.6 and 1.4 mg/kg (PMMA-trained animals) and 1.3 and 2.0 mg/kg (MDMA-trained animals) for (–)α-ET and (+)α-ET, respectively. These results indicated that the stimulant or amphetamine-like nature of α-ET resides primarily with its (–)isomer whereas hallucinogenic or DOM-like character resides primarily with the (+)isomer, and that both optical isomers of α-ET are capable of producing PMMA-like and MDMA-like stimulus effects at relatively similar doses [77]. As such, (–)α-ET represents the first indolealkylamine that might be reasonably classified as an A/P type agent, and (+)α-ET as a D/P-type agent (see Figure 6-14).

α-ET (2.5 mg/kg) was employed as a training drug in rats (ED_{50} = 1.3 mg/kg) using a standard two-lever operant paradigm [78; see also Chapter 3]. In tests of stimulus generalization the α-ET stimulus generalized to *S*(–)α-ET (ED_{50} = 1.6 mg/kg) and *R*(+)α-ET (ED_{50} = 1.3 mg/kg). Tests of stimulus generalization were also conducted with three prototypical phenylisopropylamines: (+)amphetamine, DOM, and PMMA. The α-ET stimulus generalized to DOM (ED_{50} = 0.4 mg/kg) and PMMA (ED_{50} = 0.7 mg/kg), and partially generalized to (+)amphetamine. The weak amphetamine-like character of racemic α-ET might have been overshadowed by the DOM-like nature of the (+)-isomer to disrupt the animals' behavior at a dose lower than could be attained to elicit amphetamine-like action. The results confirm that the mechanism of action of α-ET as a discriminative stimulus is likely complex, and has yet to be elucidated. For example, both isomers of α-ET are serotonin releasing agents (EC_{50} = 20 nM and 68 nM for the (+)- and (–)-isomer, respectively), whereas (+)α-ET (EC_{50} = 64 nM) is more potent than its (–)isomer (EC_{50} = 900 nM) at releasing dopamine, and neither isomer is effective at releasing norepinephrine (EC_{50} > 10,000 nM) [79]. This profile might contribute to its stimulus character and suggests that future drug discrimination studies with this agent focus on its individual optical isomers, rather than the racemate, as training drugs.

G. ANXIOLYTIC AGENTS

1. Benzodiazepines, Buspirone, and Arylpiperazines

Anxiolytic agents do not necessarily produce a common stimulus effect in animals. The arylpiperazine analog buspirone ("*BuSpar*"), initially developed as a potential antipsychotic agent, was introduced clinically some years later in the mid-1980s, as a novel anxiolytic agent (see Figure 6-17 for chemical structure). Buspirone was examined in rats trained to discriminate the anxiolytic agents oxazepam and diazepam from saline vehicle (see also: Chapter 5, section C). It was assumed, at the time, that "anxiolytic-trained" animals might recognize this novel anxiolytic agent. Interestingly, both the oxazepam and the diazepam stimulus *failed* to generalize to buspirone [80–82]. Anxiolytic agents related in structure to buspirone, that is, gepirone and ipsapirone (also known then as isapirone and TVXQ 7821) (Figure 6-17) also produced anxiolytic actions in preclinical studies (e.g., see Chapter 3). These, too, were examined in diazepam-trained animals (using a 3.0 mg/kg training dose of diazepam) and, here too,

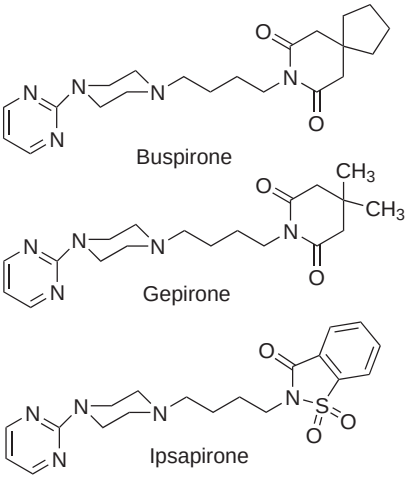


Figure 6-17. Chemical structures of the second generation anxiolytic agents buspirone, gepirone, and ipsapirone.

diazepam-stimulus generalization *failed* to occur to either of these agents [81]. Furthermore, Rosecrans and co-workers showed that buspirone-trained rats failed to recognize the benzodiazepine anxiolytic agent oxazepam [80]. However, using diazepam-trained rats, stimulus generalization occurred to a large number of other anxiolytic benzodiazepine derivatives, and generalization potencies were significantly correlated with their human anxiolytic potencies [82]. Clearly, the novel buspirone-related anxiolytic agents were not producing discriminative stimulus effects similar to those of the anxiolytic agent diazepam. Yet, like diazepam, these agents produced anxiolytic actions in other animal studies [e.g. 82; see also Chapter 3]. This was, perhaps, the first evidence that animals are not discriminating a specific overt *"anxiolytic"* effect from saline vehicle. Evidently, not all anxiolytic agents "are created equal." That is, anxiolytic agents effective in humans do not necessarily produce a "*common*" discriminative stimulus effect—at least not from the perspective of the test animals; that is, animals did not recognize a "*generalized anxiolytic*" action. The stimulus actions of these agents are very likely (i.e., must be) receptor-based.

Although buspirone's mechanism of action was unknown at the time, it was speculated to be related to its rather modest affinity for 5-HT_1 serotonin receptors. Buspirone showed higher affinity for 5-HT_1 than 5-HT_2 receptors (the only two major serotonin receptor populations then known). Subpopulations of 5-HT_1 receptors (i.e., 5-HT_{1A} and 5-HT_{1B} receptors) had just been discovered but they had yet to be extensively studied. For many years following the discovery of 5-HT_{1A} and 5-HT_{1B} receptors, arylpiperazines (such as TFMPP; see Figure 6-1 for chemical structure) were thought to be 5-HT_{1B}-selective serotonergic agents; as such, it was quite reasonably assumed that buspirone and related agents, because they, too, possessed an arylpiperazine moiety, might be working through a 5-HT_{1B} serotonin receptor mechanism. But, it was later

found that the arylpiperazine TFMPP-stimulus did not generalize to buspirone, gepirone, or ipsapirone (nor to benzodiazepine anxiolytic agents such as diazepam) [81].

It is now recognized that "*simple*" arylpiperazines (i.e., arylpiperazines lacking an N_4 substituent, such as TFMPP) are nonselective serotonergic agents. For additional discussion, see Chapter 7. Drug discrimination studies with TFMPP as training drug, as well as later radioligand binding studies, showed that simple arylpiperazines were not 5-HT_{1B}-selective serotonergic agents [83, 84]. It was also demonstrated, on the basis of drug discrimination and radioligand binding studies, that incorporation of a piperazine-ring N_4-substituent very often converted nonselective arylpiperazines to agents with greater 5-HT_{1A} receptor affinity and selectivity (i.e., agents we termed "*long-chain arylpiperazines*," or *LCAP*s). This set the stage.

The anxiolytic agents diazepam, buspirone, gepirone, and ipsapirone were examined in rats trained to discriminate 0.2 mg/kg of 8-OH DPAT (an agent eventually shown to be, primarily, a 5-HT_{1A} serotonin receptor agonist) from saline vehicle [81, 85, 86]. 8-OH DPAT-trained animals did not recognize diazepam (nor any other benzodiazepine anxiolytic agent); however, the 8-OH DPAT stimulus generalized to buspirone, gepirone, and ipsapirone (Figure 6-18). Taken together, this provided the first evidence that 1) "anxiolytic-trained" animals (i.e., rats trained to discriminate a benzodiazepine anxiolytic agent from saline vehicle) did not recognize these new anxiolytic agents, and that these new agents might be acting via a different receptor mechanism; 2) the new anxiolytic agents are acting via a mechanism different from that of diazepam-type anxiolytic agents; 3) animals don't "cue" on a specific behavioral effect, but, rather "cue" on mechanistic effects; and that 4) these new anxiolytic agents might be working through a 5-HT_{1A} serotonin receptor *agonis*t mechanism. 8-OH DPAT was later shown to be a 5-HT_{1A}-selective agonist. (Actually, 8-OH DPAT is now recognized to be a 5-HT_{1A}/5-HT_7 receptor agonist with higher affinity for the former than for the latter.) It might be noted in passing that the 8-OH DPAT stimulus was not antagonized by the benzodiazepine receptor antagonist flumazenil [81]. Today, it is thought that buspirone

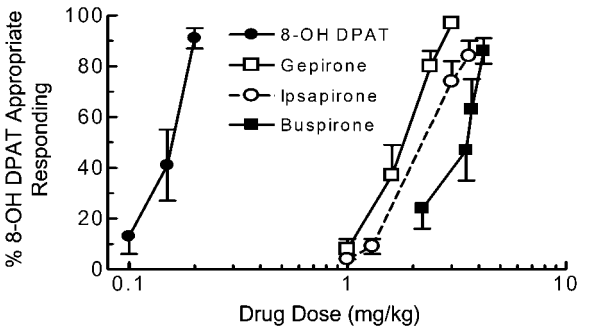


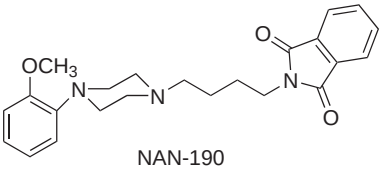
Figure 6-18. Stimulus generalization studies with rats (n = 4 to 6 per dose) trained to discriminate 0.2 mg/kg of the 5-HT_{1A} receptor agonist 8-OH DPAT from saline vehicle. Saline produced <20% drug-appropriate responding (data not shown). See text for further explanation.

and structurally related anxiolytic agents produce their actions *via* activation of 5-HT_{1A} serotonin receptors.

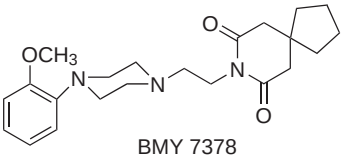
2. NAN-190 and 5-HT_{1A} Receptor Antagonists

8-OH DPAT was, on the basis of functional studies, initially considered to be a 5-HT receptor agonist and, later, upon discovery of 5-HT_{1A} and 5-HT_{1B} receptors, and on the basis of its radioligand binding and functional activity profile (conducted subsequent to the above-mentioned drug discrimination studies), to be a 5-HT_{1A} receptor agonist. What was required was a 5-HT_{1A} serotonin receptor antagonist that could attenuate the stimulus effects of 8-OH DPAT. At the time, no such agent was known. Are the stimulus effects of 8-OH DPAT, and 8-OH DPAT-stimulus generalization to buspirone, due to a 5-HT_{1A} serotonin receptor-induced agonist effect? Can an agent be developed that might serve as a selective 5-HT_{1A} serotonin receptor antagonist?

NAN-190, developed in our laboratories (see Chapter 7 for additional discussion, and Figure 6-19 for chemical structure) on the basis of radioligand binding experiments and drug discrimination studies, was the first 5-HT_{1A} serotonin receptor antagonist demonstrated to block the discriminative stimulus effects of 8-OH DPAT in animals [87]. Hence, there now was evidence to support the suggestion that the non-benzodiazepine anxiolytic agent buspirone, and related non-benzodiazepine LCAP anxiolytic agents, might act through a 5-HT_{1A} receptor mechanism. Furthermore, NAN-190 failed to block the stimulus effects of diazepam in diazepam-trained animals (Glennon and Young; unpublished data). Other studies have since corroborated these



NAN-190



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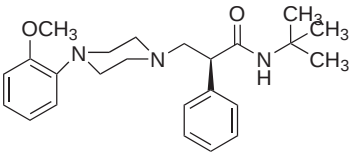

WAY 100135

Figure 6-19. Chemical structures of early 5-HT_{1A} receptor antagonists.

findings or conclusions. Greater than 75 studies of stimulus generalization and stimulus antagonism now have been conducted with the 5-HT_{1A} serotonin receptor agonist 8-OH DPAT as training drug (see Table 3-1), and additional studies have been conducted with various LCAPs as training drug. For example, Tricklebank et al. [88], using rats trained to discriminate 8-OH DPAT from vehicle, found that the 8-OH DPAT stimulus generalized to ipsapirone. Spencer and Traber [89] trained rats to discriminate ipsapirone from vehicle and found that the ipsapirone-stimulus generalized to 8-OH DPAT. It was also possible to block the 8-OH DPAT stimulus in rats with NAN-190 [89]. Barrett and Gleeson [90] demonstrated that the discriminative stimulus effects of 8-OH DPAT in pigeons could be antagonized by NAN-190, and another early 5-HT_{1A} receptor antagonist BMY 7378 (see Figure 6-19 for chemical structure); but BMY 7378 only partially antagonized the stimulus effects of 8-OH DPAT in rats [91]. Przegalinski and coworkers [92], using rats trained to discriminate 8-OH DPAT from vehicle, showed that the 5-HT_{1A} receptor antagonists NAN-190 and, the then novel 5-HT_{1A} serotonin receptor antagonist (related in structure to NAN-190), WAY 100135 (see Figure 6-19 for chemical structure), blocked the discriminative stimulus effects of 8-OH DPAT. The results of various drug discrimination studies generally agreed that these so-called "*second generation anxiolytic agents*" (or LCAPs) act through a 5-HT_{1A} receptor agonist (or partial agonist) mechanism. This is one of the first instances where drug discrimination studies assisted in determining the mechanism of action of a clinically available therapeutic agent, and demonstrated that the stimulus effects of anxiolytic agents are, indeed, "mechanism-based." That is, the stimulus effects of anxiolytic agents, as a whole, might not be related to their ability to produce a "generalized" anxiolytic effect but are related more to their specific mechanism(s) of action. In a broader sense, this means that *animals can discriminate between agents that produce a common behavioral effect* (e.g. an anxiolytic-like effect; see Chapter 3) and, more appropriately, can *discriminate between different pharmacological mechanisms of action underlying a common action*! In fact, human subjects have been trained to discriminate the stimulus effects of buspirone *versus* diazepam *versus* vehicle in a three-choice task (see Chapter 3).

General conclusion: diazepam-related benzodiazepine agents (e.g., oxazepam and diazepam) and certain non-benzodiazepine LCAPs produce an anxiolytic effect (in animals and in humans), but they very likely do so through an entirely different mechanism of action, And, animals are able to recognize these differences in tests of stimulus generalization. See Chapter 7 for further discussion of the development of NAN-190.

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7

DRUG DISCRIMINATION AND DEVELOPMENT OF NOVEL AGENTS AND PHARMACOLOGICAL TOOLS

A. APPLICABILITY AND GENERAL COMMENTS

Drug discrimination studies have played a role in drug discovery and development. There are several instances where agonists of a known central mechanism have been used as training drugs in order to develop entirely novel antagonists, or where standard

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

neurotransmitter antagonists were employed to identify the mechanism of action of a training drug—whose mechanism of action might have been hitherto unknown—leading to the eventual development of novel agents. As will be discussed below, the development of the antidiarrheal agent loperamide and the atypical antipsychotic agent risperidone represent two of the first examples of therapeutic agents whose discovery was intimately linked, at least in part, to drug discrimination studies. But other agents, some of which have eventually found their way onto the market (and many that have not), were examined in drug discrimination studies, both in the pharmaceutical industry and in academia, while they were being developed. A case in point already discussed is the anxiolytic agent buspirone (see Chapter 6, section G). Hundreds of therapeutic agents already on the market and drugs of abuse on the clandestine market, also have been examined *ex post facto* in drug discrimination studies in tests of stimulus generalization or antagonism. These studies were often conducted to investigate action (i.e., classification of related agents and/or metabolites as to whether or not they produced stimulus effects similar to a known agent) and potential mechanisms of action (see Chapter 6 for selected examples). But, the paradigm has also made a substantial contribution to the development of new agents, agonists and antagonists, as well as radioligands, as pharmacological tools. Included below are several examples selected from our laboratories but, once again, with the realization that other authors might have selected entirely different examples from the extensive body of available literature. What follows is by no means an attempt to be comprehensive or exhaustive. Rather, what is presented is meant to be representative of how the technique can be used to achieve a desired goal. Because loperamide and risperidone were among the first agents introduced clinically on the basis of drug discrimination studies, they too are described. It also should be appreciated that *drug discrimination studies, like other pharmacological studies, cannot be employed alone to fully characterize an agent*. It should become quickly apparent that drug discrimination studies, such as those described below, are typically—indeed, almost always—combined with other pharmacological investigations. As such, drug discrimination studies represent one of a battery of biological "assays" that are employed in drug development, or in the development/identification of pharmacological tools.

B. NOVEL 5-HT_2 SEROTONIN RECEPTOR ANTAGONISTS

1. Phenylalkylamines

As described in Chapter 4, the 2,5-dimethoxy substitution pattern of the phenylisopropylamine hallucinogens is important for DOM-like stimulus action and 5-HT_2 receptor affinity. An initial quantitative structure-activity relationship (QSAR) study indicated that DOM-stimulus generalization potency was related to the lipophilicity of the 4-position substituent of 2,5-DMA analogs (see Chapter 5). The nature of the 4-position substituent of these compounds was found to modulate 5-HT_2 receptor affinity over a very broad (>10,000-fold) range [1]. Holding the 2,5-dimethoxy groups of DOM constant, the influence of 4-position substituents was examined by measuring the 5-HT_2

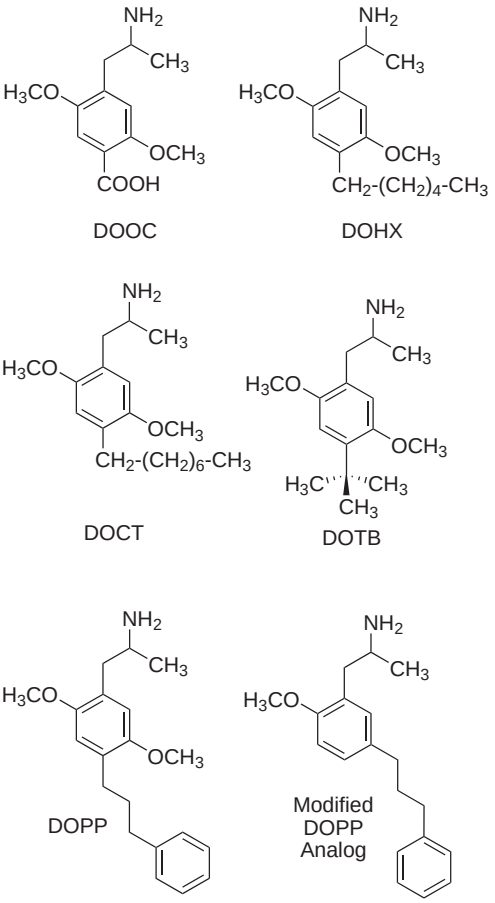


Figure 7-1. Chemical structures of the DOM metabolite DOOC, and several 4-alkyl analogs: DOHX, DOCT, DOTB, DOPP, and a modified DOPP analog.

receptor affinities of 27 4-substituted 2,5-DMA analogs. A QSAR study generated *relating equations* revealing that polar substituents were not well tolerated at the 4-position of 2,5-DMA analogs, but that 5-HT$_2$ receptor affinity increased as the hydrophobicity (i.e., lipid solubility, as denoted by the π value of a substituent) of the 4-position substituent increased [1, 2 and see Chapters 5 and 6]. For example, the polar compound, and DOM metabolite, DOOC (see Figure 7-1 for chemical structure), at doses of up to 10 mg/kg, failed to substitute in DOM-trained rats (DOM ED_{50} = 0.45 mg/kg) and produced a maximum of <10% DOM-appropriate responding; DOOC also lacked affinity (K_i > 50,000 nM) for 5-HT$_2$ receptors [1]. Hence, it could now be concluded (see Chapter 5) that in addition to the inability of DOOC to penetrate the blood-barrier, had it been able to enter the brain it very likely still would be inactive as a DOM-like agent because it does not bind at 5-HT$_2$ receptors.

Interesting, though, is that the relating equation for 5-HT_2 receptor affinity and lipophilicity was different from that identified for stimulus generalization potency and lipophilicity. That is, the latter identified both π and π^2 terms, indicating a parabolic relationship between generalization potency and lipophilicity, whereas the former lacked the π^2 term [1, 2]. In other words, 5-HT_2 receptor affinity increased as lipophilicity increased. For example, DOAM (which failed to substitute in DOM-trained animals) displayed nearly ten times higher 5-HT_2 receptor affinity than the less lipophilic DOPR (which potently substituted in DOM-trained animals).

Consequently, a series of 4-alkyl (i.e., nonpolar, lipophilic) analogs was prepared and explored. That is, 4-substituted 2,5-DMA analogs were examined where the 4-methyl group of DOM was *homologated* (i.e., where the methyl group was consecutively extended by additional methylene groups—this was discussed earlier—and some results were shown in Figure 5-7). Other *homologs* were prepared and examined including the 4-*n*-hexyl compound (i.e., DOHX), the 4-*n*-octyl homolog DOCT, and the 4-(3-phenyl)propyl congener DOPP (Figure 7-1). DOHX (K_i = 2.5 nM) and DOCT (K_i = 3.0 nM) displayed the highest 5-HT_2 receptor affinities in the series. However, consistent with results obtained with DOAM, the DOM-stimulus failed to generalize to DOHX or DOCT. If compounds bind at a receptor population but fail to act as agonists, there is a reasonable expectation that they might act as antagonists. Indeed, DOHX and DOCT were shown to dose-dependently antagonize 5-HT_2-mediated contractile actions of rat thoracic aorta induced by 5-HT [1]. DOTB (K_i = 19 nM) partially antagonized the DOM stimulus (Figure 7-2). However, doses of DOTB administered alone to DOM-trained rats produced >20% drug-appropriate responding in tests of stimulus generalization, and administration of DOTB alone or in combination with DOM severely depressed the animals' response rates (Figure 7-2) precluding examination of higher drug doses. Studies with the structurally-related DOPP (5-HT_{2A} K_i = 10 nM) [1] showed that it was a 5-HT_2 receptor antagonist in a functional (i.e., PI or phosphoinositide hydrolysis) assay, and attenuated the stimulus effects of DOM [3]. Subsequent studies revealed that DOPP, at very high concentrations, might be a low-efficacy partial agonist in the PI assay [4]. Nevertheless, a combination of drug discrimination and radioligand binding studies had identified a new structural type of 5-HT_2 receptor antagonist.

A commonly held tenet in medicinal chemistry is that *if two series of agents are binding in the same manner at a receptor, parallel changes in chemical structure often result in parallel shifts in affinity*. Given the assumption that there is no reason why agonists and antagonists must bind at a receptor in a similar fashion—although they must generally share at least one common (receptor) site of interaction in order to bind in a competitive manner—the question raised was: do phenylisopropylamine-related 5-HT_2 receptor antagonists, such as DOPP and structurally-related compounds, bind in a manner similar to DOM-related phenylisopropylamine 5-HT_2 receptor agonists? If these agents bind differently, their structure-activity relationships for binding should differ. Studies quickly showed that the 2,5-dimethoxy substitution pattern (of 2,5-dimethoxy-substituted 5-HT_2 receptor antagonists) was not important for antagonist action, or for the binding of these antagonists at 5-HT_2 receptors. Indeed, *structure-affinity relationships* for the binding of antagonist phenylisopropylamines were found

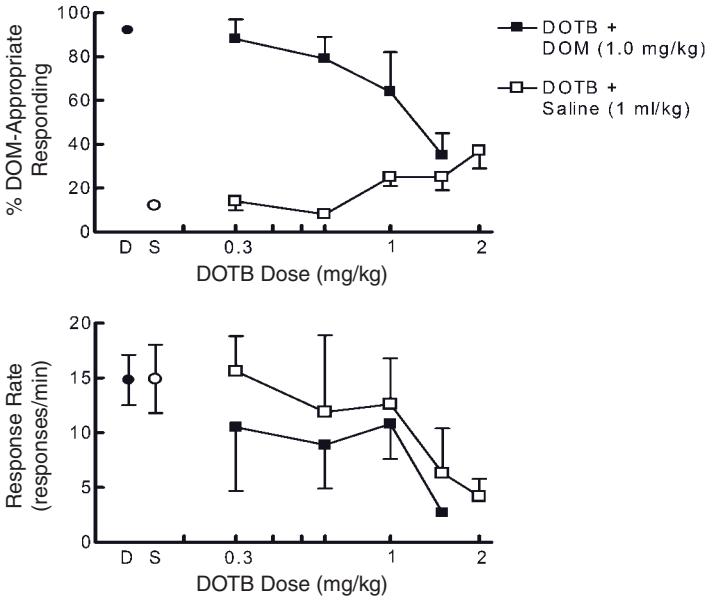
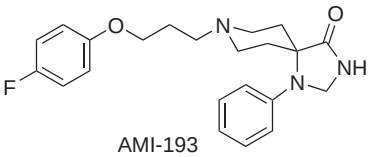


Figure 7-2. Results of stimulus generalization and antagonism studies with DOTB using rats trained to discriminate DOM (1.0 mg/kg) from saline vehicle (upper panel). D = effect of 1.0 mg/kg of DOM, and S = effect of saline vehicle. Response rates of animals administered DOTB alone or in combination with the training dose of DOM (lower panel).

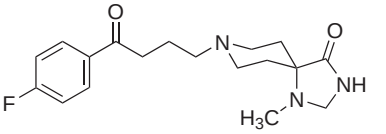
to be quite different from those with agonist action. This series of studies culminated with several high-affinity DOPP analogs (e.g., the "*modified DOPP analog*"; 5-HT$_{2A}$ K_i = 13 nM; see Figure 7-1 for chemical structure) which behaved as 5HT$_2$ receptor antagonists in a phosphoinositide hydrolysis assay [5]. Although additional pharmacological studies were conducted, no further drug discrimination studies were performed with these agents. Here is a case where drug discrimination data, radioligand binding data, and QSAR studies were jointly employed to identify and develop the first members of a novel class of 5-HT$_2$ receptor antagonists that were subsequently evaluated using other types of functional assays deemed more rapid, sensitive, and appropriate for addressing the action in question.

2. Triazaspirodecanones

Another 5-HT$_2$ receptor antagonist, AMI-193 (see Figure 7-3 for chemical structure), was discussed in Chapter 6. AMI-193, developed using the "deconstruction-reconstruction-elaboration" approach (see APPENDIX), showed >1,000-fold selectivity for 5-HT$_{2A}$ *versus* 5-HT$_{2C}$ receptors and potently blocked the discriminative stimulus effects of DOM. A related analog, KML-010 (see Figure 7-3 for chemical structure), another novel 5-HT$_2$ receptor antagonist, was found to display even greater 5-HT$_{2A}$ receptor selectivity than AMI-193. The development of both of these antagonists had their roots in drug discrimination studies [see 6 and 7 for a review].



AMI-193



KML-010

Figure 7-3. Chemical structures of the 5-HT_{2A} receptor antagonists AMI-193 and KML-010.

C. 5-HT_2 SEROTONIN RECEPTOR AGONISTS AND RADIOLIGANDS

Soon after the discovery of the 5-HT_2 population of serotonin receptors it was proposed that these receptors might exist in two affinity-states: one state having high affinity for agonists, and the other having reduced affinity for agonists. Other investigators proposed that these might represent two distinct 5-HT_2 receptor subtypes. What was required was a high-affinity agonist radioligand. Possible candidates that were considered initially included such compounds as DOHX or DOCT ($K_i \leq 3$ nM)—DOM analogs that displayed substantially higher affinity than DOM (K_i *ca.* 100 nM) for 5-HT_2 receptors—that made them amenable to conversion to radioligands. However, the results of stimulus generalization studies and other pharmacological investigations demonstrated that DOHX and DOCT were either, at best, 5-HT_2 receptor partial agonists or, more likely, 5-HT_2 receptor antagonists. Hence, they were excluded from consideration for labeling [2].

On the basis of SAR and mechanistic studies (see Chapters 5 and 6, respectively), DOB (K_i = 49 nM) and DOI (K_i = 19 nM), the bromo and iodo counterparts of DOM, were next considered for possible radiolabeling. DOB and DOI displayed higher affinities than DOM for 5-HT_2 receptors, and DOI was particularly attractive because it could be converted to a radioiodinated ligand (i.e., a radioligand with higher specific activity than a tritiated radioligand). Stimulus generalization studies using DOM-trained animals revealed that both DOB and DOI produced DOM stimulus effects with their *R*-isomers being more potent than their racemates (see Figure 7-4 for selected results). [^{3}H]DOB and [^{125}I]DOI were subsequently prepared and investigated [8-11].

Today, both [^{3}H]DOB and [^{125}I]DOI are commercially available for radioligand binding and autoradiographic studies, and [^{123}I]DOI and *R*(-)[^{123}I]DOI have been explored as SPECT imaging agents [7, 12]. Results of a typical experiment using [^{123}I] DOI are sown in Figure 7-5. These radioligands were developed as a direct consequence of drug discrimination studies.

Thus, an undeniable link had been forged between the actions of classical phenylalkylamine hallucinogens and a 5-HT_2 (5-HT_{2A}) receptor agonist mechanism. The

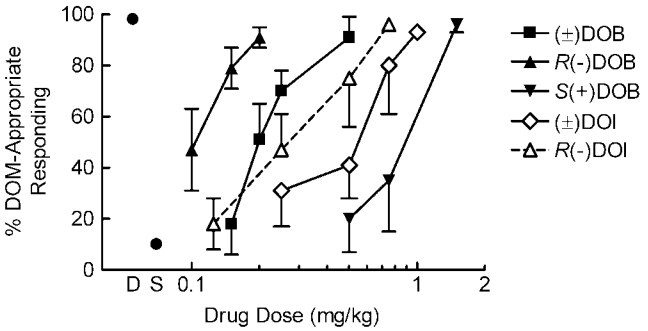


Figure 7-4. Results of stimulus generalization studies with DOB, DOI, and selected isomers in rats trained to discriminate 1.0 mg/kg of DOM from saline vehicle. D = response elicited by the training dose of DOM; S = response elicited by saline vehicle.

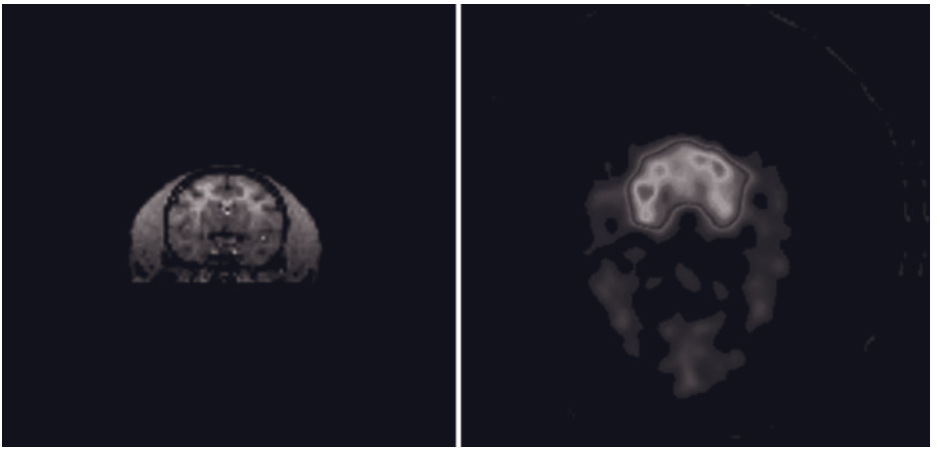


Figure 7-5. Results of a typical experiment where [^{123}I]DOI was injected into a Rhesus monkey and imaged by SPECT as described in the text [12]. The image was taken 4 h post-injection and indicates accumulation of specific [^{123}I]DOI binding in the cortical region of the brain. MRI indicated the same brain region of the coronal SPECT viewed through the basal ganglia. (Unpublished photo courtesy of Dr. Kan Sam Lee, NIH.) (See also color insert.)

possibility of agonist-directed trafficking (i.e., regulation of intracellular effectors) remains to be fully explored and offers some new research directions with regard to these agonists [13]. But, might it be possible to develop novel 5-HT$_2$ (5-HT$_{2A}$) receptor agonists with therapeutic potential despite the obvious shortcomings (i.e., hallucinogenic side effects) associated with 5-HT$_{2A}$ agonism? That is, is it worthwhile examining novel 5-HT$_2$ agonists for possible therapeutic utility?

Activation of 5-HT$_{2A}$ receptors in the eye results in a reduction of intraocular pressure that might be beneficial in the treatment of glaucoma. Given the low drug

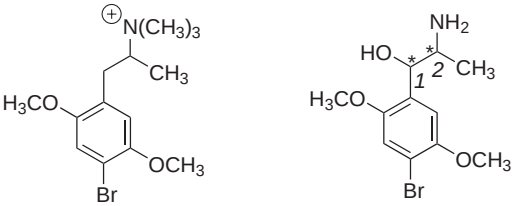


Figure 7-6. Chemical structure of QDOB and β-hydroxy DOB (i.e., β-OH DOB). The asterisk denotes a chiral center.

concentrations that would be applied, and an intraocular route of administration, relatively little of the agent would likely penetrate the blood-brain barrier to produce central side effects. Consequently, development of novel 5-HT_{2A} receptor agonists with reduced lipophilicity would further decrease the likelihood of brain penetration. Using DOB as a template, how can the lipophilicity of DOB be reduced? One strategy would be to quaternize the terminal amine of DOB because quaternary amines do not readily penetrate the blood-brain barrier. However, SAR studies of the type described in Chapter 5 showed that QDOB, the *N*,*N*,*N*-trimethyl quaternary amine analog of DOB (see Figure 7-6 for chemical structure), does not substitute in rats trained to discriminate *R*(-)DOB from saline vehicle, nor does it bind to 5-HT_{2A} receptors [14]. Another general strategy to decrease the lipophilicity of DOB-type agents (and of CNS-active agents in general) is to introduce a polar substituent (e.g., to replace the 4-bromo group of DOB with a 4-carboxylic acid or it's salt). But, as already shown (see section A above), the carboxylic acid counterpart of DOB (i.e., DOOC; see Figure 7-1), neither produces drug-appropriate stimulus effects in rats, nor does it bind to brain 5-HT_{2A} receptors. A different strategy was required. The only position of the DOB molecule that had not been previously investigated up to this time was the benzylic position (i.e., β- or 1-position; see Figure 7-6), and introduction of a polar hydroxyl group at this position should, expectedly, reduce lipophilicy. But, will β-hydroxy DOB (i.e., β-OH DOB) retain affinity for 5-HT_{2A} receptors? Another problem was that introduction of a β-hydroxyl group creates a second chiral center and four optical isomers are possible (for chemical structures see Figure 7-6).

All four isomers of β-hydroxy DOB (1*S*,2*R*; 1*R*,2*S*; 1*S*,2*S*; 1*R*,2*R*) were synthesized and evaluated [15]. Only 1*R*,2*R*-β-OH DOB displayed the 5-HT_{2A} receptor affinity (K_i = 0.5 nM) and intrinsic efficacy of DOB. Furthermore, topical administration of this isomer reduced intraocular pressure in a monkey assay [15]. Given the receptor binding and efficacy properties of 1*R*,2*R*-β-OH DOB, it should substitute in DOM-trained animals; however, if 1*R*,2*R*-β-OH DOB is less brain penetrant than DOB (as proposed), it should be less potent than DOB. Indeed, in tests of stimulus generalization using DOM-trained rats, 1*R*,2*R*-β-OH DOB was substantially less potent (ED_{50} = 1.4 mg/kg; 4.3 μmoles/kg) than DOM, and >15 times less potent than *R*(-)DOB (0.09 mg/kg; 0.25 μmoles/kg) [15]. As such, and given its proposed (intraocular) route of administration, 1*R*,2*R*-β-OH DOB might find use for the treatment of glaucoma with negligible CNS side effects.

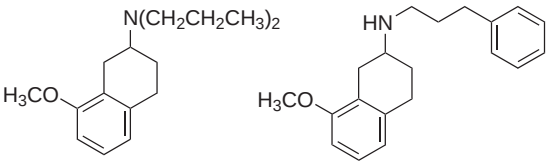


Figure 7-7. Chemical structures showing the similarity between 8-OMe DPAT and NAN-360.

D. AMINOTETRALINS AS 5-HT$_{1A}$ SEROTONIN RECEPTOR LIGANDS

As discussed in Chapter 5, 8-OH DPAT was identified as a novel serotonergic agonist in the early 1980s. Later, it was shown that 8-OH DPAT was a 5-HT$_{1A}$ receptor agonist. In fact, 8-OH DPAT represented the first serotonin receptor agonist with selectivity for a specific 5-HT receptor subpopulation. With the availability of 8-OH DPAT-trained animals (see Chapter 5) it was possible to utilize drug discrimination studies to identify novel agonists (in tests of stimulus generalization) and potential antagonists (in tests of stimulus antagonism). And, it might be noted that no 5-HT$_{1A}$-selective serotonin receptor antagonists were available at that time. Using [^{3}H]8-OH DPAT as a radioligand, 5-HT$_{1A}$ receptor affinities could now be measured directly. Interestingly, DPAT analogs with large terminal amine groups retained affinity for 5-HT$_{1A}$ receptors even though they did not produce 8-OH DPAT-like stimulus effects in animals (e.g., 8-OH DBAT) (see Chapter 5). This suggested that such compounds might behave as potential 5-HT$_{1A}$ receptor antagonists.

The first question addressed was whether or not the hydroxyl group of 8-OH DPAT was required for 5-HT$_{1A}$ receptor binding and 8-OH-DPAT-like stimulus effects. *O*-Methylation of 8-OH DPAT ($K_i = 1.2$ nM), to afford 8-OMe DPAT ($K_i = 1.3$ nM), resulted in a compound to which the 8-OH DPAT stimulus generalized ($ED_{50} = 0.22$ mg/kg) [16]. Armed with this information, it was subsequently demonstrated that 8-methoxy-2-aminotetralins retained affinity for 5-HT$_{1A}$ receptors even when they possessed fairly large *N*-alkyl groups (e.g., see NAN-360, $K_i = 2.5$ nM; Figure 7-7) [17]. However, no additional stimulus antagonism studies were conducted with this series of compounds once they were determined to be low-efficacy partial agonists rather than full antagonists in an adenylate cyclase assay [18].

Continued investigation eventually might have resulted in antagonist analogs but, in fact, this project was abandoned in favor of another, concurrent, 5-HT$_{1A}$ receptor antagonist project (see Arylpiperazines below).

E. ARYLPIPERAZINE 5-HT$_{1A}$ SEROTONIN RECEPTOR ANTAGONISTS

Certain arylpiperazines, such as TFMPP (for chemical structure see Figure 7-8), were initially identified as "serotonin receptor agonists" and later as "serotonin receptor agonists producing stimulus effects distinct from those of DOM and 8-OH DPAT," and

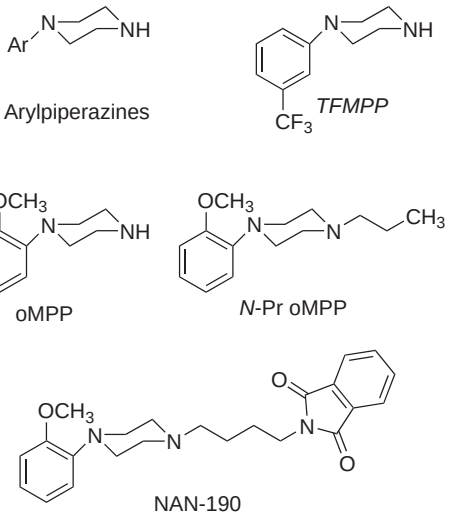


Figure 7-8. Chemical structures of various arylpiperazines including TFMPP, oMPP and N-propyl oMPP, which aided the discovery of the 5-HT_{1A} receptor antagonist NAN-190.

yet later, following the discovery of 5-HT_1 and 5-HT_2 receptors, as "5-HT_1 serotonin receptor agonists." During this period, it was demonstrated that TFMPP and 8-OH DPAT produced different stimulus effects regardless of which of the two was used as the training drug. That is, a TFMPP stimulus did not generalize to 8-OH DPAT, and an 8-OH DPAT stimulus did not generalize to TFMPP. Following the discovery of 5-HT_1 and 5-HT_2 receptors these findings were difficult to reconcile because both TFMPP and 8-OH DPAT displayed affinity for 5-HT_1 receptors but possessed little affinity for 5-HT_2 receptors.

Subsequently, 5-HT_{1A} and 5-HT_{1B} receptors were identified and, because 8-OH DPAT was eventually shown to be a 5-HT_{1A} receptor agonist, TFMPP was considered, by default, to be a 5-HT_{1B} receptor agonist. This *misconception*, held by many investigators at that time, was considered gospel for several years. However, it was later demonstrated, on the basis of drug discrimination studies, that various arylpiperazines, including TFMPP, were *non-selective* serotonergic agents [19]. Nevertheless, it was realized early on that TFMPP and 8-OH DPAT produced distinctly different stimulus effects in animals.

A structure-activity study was undertaken to determine the structural requirements for arylpiperazines to produce TFMPP-like stimulus effects in rats. TFMPP displayed 7-fold selectivity for 5-HT_{1B} (K_i = 25 nM) versus 5-HT_{1A} receptors. 1-(2-Methoxyphenyl) piperazine (commonly referred to as oMPP; see Figure 7-8 for chemical structure) was one of the first arylpiperazines to display receptor-selectivity reversal, small though it might have been, for 5-HT_{1A} versus 5-HT_{1B} receptors (5-HT_{1B} K_i = 120 nM; 5-HT_{1A} K_i = 68 nM). Nevertheless, the TFMPP stimulus generalized to oMPP. However, conversion of an arylpiperazine from a secondary amine to a tertiary amine decreased its 5-HT_{1B} receptor affinity more so than 5-HT_{1A} receptor affinity. For example, the *N-n-*

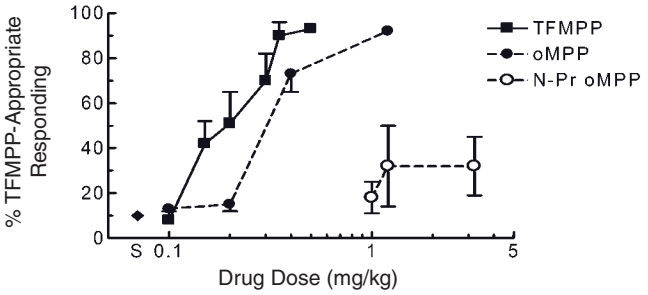


Figure 7-9. Results of stimulus generalization studies with rats trained to discriminate 0.5 mg/kg of TFMPP from saline vehicle. S = effect of saline. Administration of N-Pr oMPP doses > 3.2 mg/kg (i.e., 3.4, 4.0, and 5.0 mg/kg) resulted in behavioral disruption [20].

propyl analog of oMPP (i.e., *N*-Pr oMPP; Figure 7-8) displayed substantial selectivity for 5-HT_{1A} (K_i = 68 nM) versus 5-HT_{1B} (K_i = 5,300 nM) receptors; more importantly, unlike oMPP, *N*-Pr oMPP did not substitute in TFMPP-trained animals (Figure 7-9) [20].

Chain extension, and other structural modifications of *N*-Pr oMPP, led to novel arylpiperazines with high (i.e., sub-nanomolar) affinity for 5-HT_{1A} receptors [21]. One of these compounds (NAN-190; 5-HT_{1A} K_i = 0.6 nM; see Figure 7-8 for chemical structure) unexpectedly displayed 5-HT_{1A} receptor *antagonist* action in cellular assays and was subsequently examined alone, and in combination with 8-OH DPAT, in tests of stimulus generalization and antagonism employing 8-OH DPAT-trained rats [22]. NAN-190 failed to substitute for 8-OH DPAT and, indeed, was the first arylpiperazine 5-HT_{1A} receptor antagonist to effectively antagonize the discriminative stimulus effects of 8-OH DPAT.

A binding profile obtained for NAN-190 revealed that it was a fairly selective serotonergic agent with >100-fold selectivity for 5-HT_{1A} versus 5-HT_{1B} receptors, but that it also showed high affinity for α_1-adrenoceptors [22]. In order to eliminate a possible role for α_1-adrenoceptors in the stimulus actions of 8-OH DPAT, it was demonstrated that the α_1-adrenoceptor antagonist prazosin failed to antagonize the stimulus effects of 8-OH DPAT as training drug [22].

Stimulus antagonism studies were conducted with a variety of other *long-chain arylpiperazines* (LCAPs) and the results have been reviewed [23]. Other findings with NAN-190 or related antagonists implicating a role for 5-HT_{1A} receptors in the stimulus effects produced by 8-OH DPAT and agents such as buspirone and ipsapirone were discussed above (see Anxiolytic Agents in Chapter 6). Thus, drug discrimination studies led to some of the first examples of 5-HT_{1A}-selective antagonists.

F. MD-354 (*META*-CHLOROPHENYLGUANIDINE): A 5-HT_3 SEROTONIN RECEPTOR AGONIST

As mentioned earlier, the first three populations of serotonin receptors to be identified were the 5-HT_1, 5-HT_2, and 5-HT_3 receptors (as already noted, other populations have

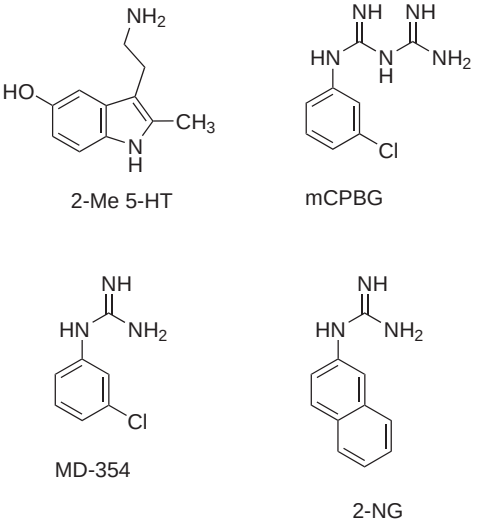


Figure 7-10. Chemical structures of agents with demonstrated 5-HT_3 agonist action: 2-Me 5-HT, mCPBG, MD-354, and 2-NG.

since been identified). A number of structurally diverse 5-HT_3 receptor antagonists were reported in fairly quick succession by the pharmaceutical industry, but very few 5-HT_3 receptor agonists were known at the time. Although displaying relatively low affinity for 5-HT_3 receptors, one of the first "selective" 5-HT_3 receptor agonists was 2-methyl 5-HT (2-Me 5-HT or 2-methylserotonin; 5-HT_3 K_i *ca*. 1,000 nM; see Figure 7-10 for chemical structure). Despite the expected low lipophilicity of 2-Me 5-HT (because it possesses a phenolic hydroxyl group and a primary amine) and, hence, its recognized inherent difficulty in possibly penetrating the blood-brain barrier, attempts were made to train rats to discriminate this agent from saline vehicle (see Chapter 3). Briefly, the objective of the investigation was that *if* stimulus control of behavior could be established by 2-Me 5-HT, studies would be conducted to determine if the agent was acting through a 5-HT_3 receptor-based mechanism. 2-Me 5-HT-trained animals might then also be employed to assess the actions of other potential 5-HT_3 agonists and antagonists. Over a period of seven months, various intraperitoneal training doses (i.e., 1.0, 2.0, 3.0, and 5.0 mg/kg with a 15-minute PSII) were explored in a (i.e., the same) group of rats and a 2-Me 5-HT dose of 5.0 mg/kg was eventually shown to serve as an effective discriminative stimulus (ED_{50} = 2.6 mg/kg) [24]. The 2-Me 5-HT stimulus generalized to another, higher-affinity, 5-HT_3 receptor agonist, *meta*-chlorophenylbiguanide (*m*CPBG, Figure 7-10; ED_{50} = 1.6 mg/kg) [24] that had just been identified. It might be noted that route of administration (and/or, perhaps, training dose or PSII) is important for the stimulus control of behavior by 2-Me 5-HT in rats. That is, a decade following this investigation, Olivier et al. [25] were unable to train rats to discriminate 2-Me 5-HT doses of up to 4.0 mg/kg (p.o.) using a 60-minute PSII. They concluded, on the basis of these, and related, studies, that 5-HT_3 receptor agonists might not be capable of producing a discriminative stimulus effects in rats [25]. But, the doses they

employed, as well as their route of administration (i.e., p.o.—and tryptamines, particularly primary amines, are typically unstable via the p.o. route of administration) might have influenced their results.

Contrary to their conclusion, 2-Me 5-HT had obviously served as a discriminative stimulus in rats at an intraperitoneal training dose of 5.0 mg/kg. Additional evidence that the 2-Me 5-HT stimulus was centrally- and 5-HT_3 receptor-mediated was that the 5-HT_3 receptor antagonist tropisetron (previously known as ICS 205-930; AD_{50} = 1 µg/kg) potently blocked the stimulus actions of 2-Me 5-HT whereas the quaternary amine analog tropisetron methiodide (see Figure 7-11 for chemical structure), a 5-HT_3 receptor antagonist that does not readily penetrate the blood-brain barrier, failed to block the effect at 10,000 times the ED_{50} dose of tropisetron [24; see Chapter 3]. Later studies, with another group of animals trained to discriminate the same dose of 2-Me 5-HT from vehicle, showed that the effect of 2-Me 5-HT could be blocked by the 5-HT_3 receptor antagonist zacopride (see Figure 7-11 for chemical structure) and its isomers (Figure 7-12), with potencies comparable to their relative affinities for 5-HT_3 receptors, that is, *S*(-)zacopride (AD_{50} = 0.05 µg/kg) > (±)zacopride (AD_{50} = 0.6 µg/kg) > *R*(+)zacopride (AD_{50} = 1.6 µg/kg), and the affinity of *S*(-)zacopride has been reported to be between 10 to 40 times that of *R*(+)zacopride [26].

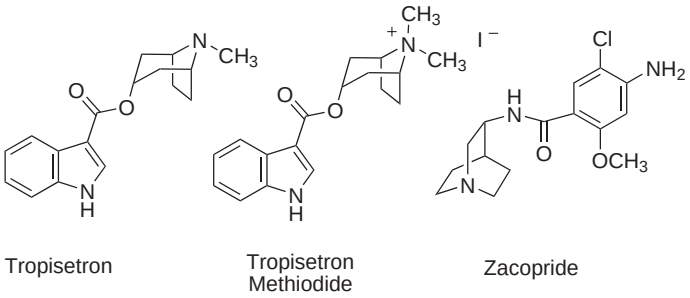


Figure 7-11. Chemical structures of several 5-HT_3 receptor antagonists.

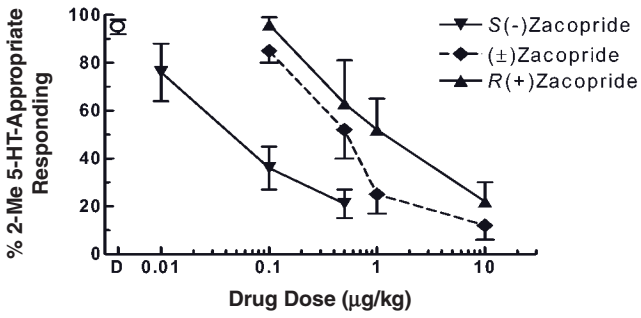


Figure 7-12. Antagonism of a 2-methyl 5-HT (2-Me 5-HT) stimulus by the zacopride and its isomers. Saline produced 8% drug-appropriate responding, and zacopride and its isomers, administered in combination with saline rather than 2-methyl 5-HT, produced <20% drug-appropriate responding (data not shown).

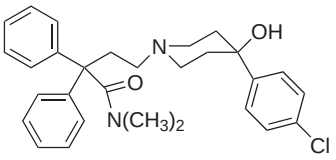
Several years later, it was found that 2-Me 5-HT binds at 5-HT_6 serotonin receptors with 30-fold higher affinity than it displays for 5-HT_3 receptors [27]; it was not shown, however, if 2-Me 5-HT was a 5-HT_6 receptor agonist or antagonist. In any event, 2-Me 5-HT could no longer be considered "selective" for 5-HT_3 serotonin receptors. Although there was little reason to question the findings of the aforementioned drug discrimination studies using 2-Me 5-HT as training drug because of the results of stimulus generalization and antagonism studies, it would certainly be reassuring to train animals to discriminate another example of a 5-HT_3 receptor agonist. Indeed, *m*CPBG (15 mg/kg, i.p.) was subsequently established as a training drug in rats [28]; however, the animals' response rates were severely depressed following administration of the training dose of training drug.

Following its discovery, the structure of *m*CPBG was *deconstructed* to determine what portions of the molecule were required for receptor binding and agonist action. The studies culminated with the identification of 3-chlorophenylguanidine (MD-354; Figure 7-10) [29]. MD-354 (training dose = 2.0 mg/kg; ED_{50} = 0.5 mg/kg) served as an effective training drug in rats [30]. The MD-354 stimulus generalized to the 5-HT_3 receptor agonists 2-naphthylguanidine (2-NG, Figure 7-10; ED_{50} = 0.7 mg/kg), *m*CPBG (ED_{50} = 1.4 mg/kg), and 2-Me 5-HT (ED_{50} = 4.5 mg/kg) [30]. Furthermore, the MD-354 stimulus was potently antagonized by the 5-HT_3 receptor antagonists (±)zacopride (AD_{50} = 0.03 mg/kg) and tropisetron (AD_{50} = 0.02 mg/kg), but not by the quaternary amine tropisetron methiodide [30].

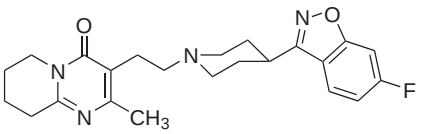
The results of these (and other pharmacological) studies [e.g., 31] identified MD-354 as a novel 5-HT_3 receptor agonist.

G. LOPERAMIDE AND RISPERIDONE: CLINICAL SUCCESSES

Perhaps the very first instances where drug discrimination studies contributed to the eventual clinical introduction of a new drug entity involves the antidiarrheal agent loperamide (Imodium®) (see Figure 7-13 for chemical structure). Janssen Pharmaceutica,



Loperamide



Risperidone

Figure 7-13. Chemical structures of loperamide and risperidone.

instrumental in the development of the opioid analgesic fentanyl, recognized that opioids generally decrease gastrointestinal motility. They found, using animals trained to discriminate fentanyl from vehicle, that the stimulus generalization potencies of various opioids were not directly correlated with their antidiarrheal potencies. This led to the idea that it might be possible to divorce antidiarrheal activity from their analgesic action (and, presumably, abuse potential) of opioids. Using antidiarrheal assays to identify novel agents that possessed the desired target action, and drug discrimination studies to identify those agents that lacked fentanyl-like stimulus character, loperamide was discovered [32; see also Chapter 16 by Colpaert].

Another agent directly stemming from drug discrimination studies conducted at Janssen Pharmaceutica was the "atypical" antipsychotic agent risperidone (Risperdal®). Given that certain antipsychotic agents behave primarily as dopamine (likely D_2) receptor antagonists, but also as serotonin (more specifically, 5-HT_2) receptor antagonists, various pharmacological studies were conducted to develop a novel antipsychotic agent. Available antipsychotic agents were relatively ineffective at blocking the stimulus effects of LSD at doses that did not impair animal behavior. Several serotonin receptor antagonists evaluated, although effective in this regard, resulted in partial substitution in the LSD-trained animals. Subsequent studies identified pirenpirone, and later risperidone (Risperdal®) (see Figure 7-13 for chemical structure), as novel agents that antagonized the LSD stimulus without eliciting any substitution at higher drug doses. These two antagonists also possessed some dopamine antagonist character. Risperidone was introduced in the early 1990s as a structurally novel atypical antipsychotic agent [reviewed: 33; see also Chapter 16 by Colpaert].

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APPENDIX

The "*Deconstruction—Reconstruction—Elaboration*" Approach

A method frequently employed by medicinal chemists to improve upon the actions of a pharmacologically active agent is to apply the *deconstruction—reconstruction—elaboration* approach [e.g., 1, 2] or a variant thereof. In this manner, the influence of various substituent groups of a particular agent on the action of that agent can often be determined.

Consider, for example, the hypothetical agent shown in Figure A-1. The molecule consists of a general (often arbitrary) core structure with appended substituents *A*-*D*. What is the influence of substituents *A*-*D* on a given "action" (e.g., potency, receptor affinity, receptor selectivity, functional activity, metabolism, distribution, undesirable side effects, toxicity)? In the *deconstruction* process, these molecular substituents are "removed" (e.g., replaced by a hydrogen atom) one at a time so that their impact on the action of interest can be measured. For example, in Chapter 5 to determine if the 4-methyl group of DOM was a requisite substituent for producing DOM-like stimulus effects in rats, the 4-methyl group was removed (i.e., replaced by –H) to afford 2,5-DMA. That is, it might be considered (referring to Figure A-1) that *D* = the terminal amine, *A* and *C* = the two methoxy groups, and *B* = the 4-methyl group of DOM. 2,5-DMA, which might be considered the *C*-*D*-*A* analog of DOM, was found to produce DOM-like stimulus effects, but with 10-fold reduced potency (see Figure 5-2). In this manner, the role of the *B*-substituent (i.e., the 4-methyl group) of DOM on DOM-like stimulus action was determined. This is simply an example. Likewise, this approach was used in deconstructing the structure of nicotine (e.g., opening of the pyrrolidine ring) and 8-OH DPAT (e.g., shortening of the di-*n*-propyl chain) (see Chapter 5).

In some instances, an atom or substituent might be replaced by something other than a hydrogen atom. For example, is the pyridine nitrogen atom important for the action of nicotine [3], are the imidazolone nitrogen atoms important for the action of spiperone [4], is the indolic nitrogen atom required for the actions of tryptamines [5]? Replacement of the ring nitrogen atom by the appropriately substituted carbon atom might answer these questions.

In the hypothetical example, it might be found that removal of substituent *A* results in an analog with enhanced potency, whereas removal of substituent *C* results in

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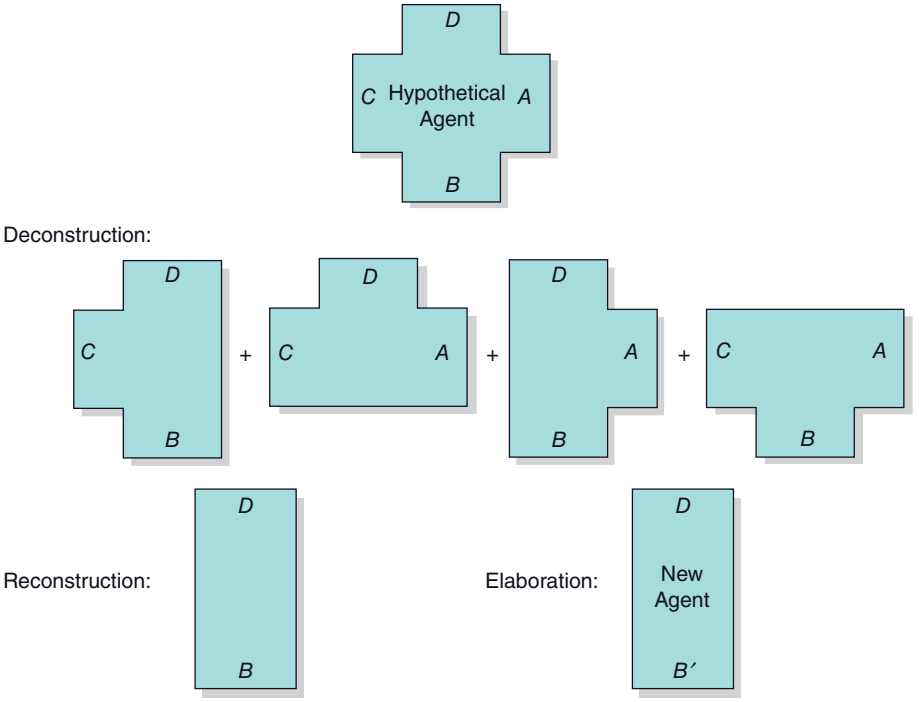

Figure A-1.

improved selectivity. Thus, a new analog lacking both of these substituents might be a more potent and selective agent relative to the hypothetical parent agent. In the *reconstruction* process, a new analog lacking substituents *A* and *C* can be synthesized and evaluated. Notice that up to this point, no new substituents have been added to the parent molecule. Assuming that this reconstructed analog displays the desired properties, the *elaboration* process can be initiated. If it had been determined during the deconstruction process that substituent *B* is critical for the action of interest, this substituent might be randomly replaced by new substituents. Unlimited numbers of substituents could be explored. Alternatively, a more systematic, less costly, and more efficient approach can be undertaken by application of the Topliss Tree [6], the Craig Plot [7], or some other related method. The latter approaches attempt to identify "why" a particular substituent is important. For example, is it the electronic character, lipophilicity, steric nature, and/or shape (e.g., length, width) of the substituent that contributes to the observed action? This process can be aided by quantitative structure-activity relationship (QSAR) studies. So, ideally, the elaboration process can utilize these concepts to minimize the number of targets required for synthesis and evaluation. An approach similar to this was employed in, for example, exploring the 4-alkyl homologs and 4-halogenated analogs (e.g., DOB) of DOM in receptor binding and drug discrimination studies (see Chapters 6 and 7). The 5-HT_{2A} receptor antagonists AMI-193 (Figure

6-4) and the "modified DOPP analog" as shown in Figure 7-1 also were developed using the *deconstruction—reconstruction—elaboration* approach.

There are occasions where this approach has worked quite well. However, there are caveats. For example, removal of one or more substituents from a parent agent might alter the manner (i.e., orientation) in which a molecule interacts with a receptor [e.g., see reference 5]. Or, it might convert, say, an agonist to an antagonist, or an antagonist to an agonist. Furthermore, an early fundamental assumption of molecular pharmacology and QSAR studies was that a given structural feature always contributes a constant amount to overall binding energy [8]. But, this was questioned many years ago by Lehmann [8] who suggested that the contribution of substituents can increase with overall affinity. Nevertheless, the approach can be effective as long as these caveats are kept in mind. Furthermore, the caveats might be inconsequential to drug discrimination studies where the questions being asked are: does compound X substitute for compound Y, or does compound X antagonize the stimulus effects of compound Y. For other examples of the application of this approach, the reader is referred to several recent reviews [1–4].

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PART II

Part II consists of invited chapters from several investigators well established in the field of drug discrimination. Chapter 8 by Jarbe is an extensive review of cannabinoid-related agents, as a particular drug class, and their effects as discriminative stimuli that highlight many of the principles described in Part I of this book. Receptor antagonists are less frequently encountered than receptor agonists as training drugs (see Part I). In Chapter 9, Porter describes the results of studies that use various neurotransmitter receptor antagonist-trained animals. In most drug discrimination studies, subjects are trained to differentiate the effects of a dose of drug *versus* vehicle conditions, but in Chapter 10 Stolerman reviews and explores studies in which discriminations involve a mixture of doses of drugs *versus* vehicle (AND-discrimination), a dose of one drug *versus* a dose of another drug (OR-discrimination), or a mixture of doses from two drugs *versus* each dose of each drug separately (AND/OR-discrimination). In Chapter 11, Negus and Banks argue that many accomplishments of drug discrimination studies can be traced to the inclusion of "choice" in experimental methodology and that this factor should be employed more often in studies of drug reinforcement or self-administration. Chapter 12, by Shelton and Balster, reviews methodological details and results from drug discrimination studies that employed inhalants as training agent or test drug. In Chapter 13, Li et al. discuss the relationships between discriminative stimulus effects, drug dependence, and withdrawal symptoms in rhesus monkeys. A general overview of human drug discrimination methodology is provided by Rush and colleagues in Chapter 14, which is followed by Perkins' description of human drug discrimination studies with nicotine in Chapter 15. Finally, Chapter 16 by Colpaert provides cogent commentaries and insights of drug discrimination studies over a 40-year career in this field.

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

8

PERCEPTUAL DRUG DISCRIMINATIVE ASPECTS OF THE ENDOCANNABINOID SIGNALING SYSTEM IN ANIMALS AND MAN

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

A. INTRODUCTION

This chapter addresses the discriminative stimulus (DS) functions of agents that primarily affect what is commonly known today as the endocannabinoid signaling system (ECS). Drug discrimination is a behavioral technique where animals or humans are trained to recognize the effects resulting from the administration of a particular drug dose of training drug and to differentiate these effects from a nondrug (or another drug) condition by responding differentially to the presence and absence of the training drug effects. Once subjects reliably discriminate between the training conditions by emitting different responses in the presence and absence of the particular training drug, new doses and drugs are introduced during test sessions that are interspersed between the regular training (or maintenance) sessions. There are essentially two outcomes from such test sessions: either the subject performs the response that had been associated with the training drug or the alternative response is emitted. In the former case, the effect(s) of the test condition is viewed as "substituting for" or "being generalized with" the training drug. If the alternate response is emitted during the test, this indicates lack of substitution or generalization and is taken as evidence that the effects resulting from the test drug probe are dissimilar to those associated with the training drug. Thus, discrimination and generalization are two basic concepts in any drug discrimination study. Essentially, therefore, discrimination refers to differences between one stimulus value along the same or other pharmacological dimensions. Generalization may be considered the inverse of discrimination. That is, the extent to which other stimulus values are "perceived" by the subject as being similar to, or the degree by which they match, the training or reference stimulus. To better understand the breadth and width of potential targets, a brief synopsis of the general characteristics of the ECS is outlined before focusing particularly on the DS functions of probes that have been used in efforts to gain further insights into the workings of this widespread bodily retrograde feedback system. Previous overviews with focus on discriminative stimulus functions of cannabinergics are found in [1–11].

B. BRIEF SYNOPSIS OF THE ENDOCANNABINOID SIGNALING SYSTEM (ECS)

The plant *Cannabis sativa* L. and its C_{21} terpenophenolic cannabinoids have been known since ancient times both for their medicinal and recreational properties [12]. In the 1960s, (-)-delta-9-tetrahydrocannabinol [Δ^9-THC; in earlier reports sometimes also referred to as (-)-Δ^1-THC] was isolated and identified as the most psychoactive component of marijuana and hashish intoxication, the chemical structure of Δ^9-THC was determined, and routes of synthesis were identified [13, 14]. Subsequent investigations led to the pharmacological characterization of cannabinoids, and indicated that they have a unique pharmacological profile [15, 16]. A major breakthrough for understanding the pharmacological profile of cannabinoid activity was the identification of a binding site named cannabinoid receptor-1 (CB_1R) for Δ^9-THC and the subsequent mapping of the binding site's distribution in the CNS [17–19]. The CB_1R is phylogenetically old and its localization within the brain would seem to fit current knowledge about the neuropharmacology of cannabinoids [20–22]. The second firmly established Δ^9-THC-receptive site is cannabinoid receptor-2 (CB_2R), likely primarily related to immunological function [23, 24]. Although previously thought to be exclusively peripheral in location [25], recent evidence suggests that CB_2R may be expressed in the central nervous system [26] even under normal physiological conditions. Additional receptor sites for cannabinergics have been postulated but are not firmly established. Adding to the complexity of the mechanism of action of examined ligands affecting the ECS is that not only do they act by activating (or inactivating) CB_1R / CB_2R, but they also exert effects at gap junctions, calcium channels that vary between ligands [27]. Further, accumulating evidence suggests that structurally dissimilar CB_1R agonists differentially regulate G-protein coupling (see [28] for review) resulting in subtle, yet different, signaling mechanisms that exhibit different downstream pharmacological profiles.

Another major breakthrough in understanding the psychopharmacology of cannabis was isolating and determining the structure of endogenous ligands for the Δ^9-THC receptor of which *N*-arachidonoylethanolamine (anandamide; AEA) was first identified [29]. This was soon followed by the discovery of other fatty acid ethanolamide brain constituents such as homo-γ-linolenoylethanolamide and *N*-docosatetraenoylethanolamine [30] and, later, 2-arachidonoylglycerol (2-AG) [31, 32; for an overview see 12]. These ligands (i.e., AEA and 2-AG) behave, respectively, as partial and full agonists at CB_1R and CB_2R but their specific role(s) in maintaining homeostasis is only now beginning to emerge. Unlike most other neurotransmitters, the endocannabinoids are *not* stored for release but, rather, are synthesized "*on demand*," and one major role for endocannabinoids appears to be in regulating neurotransmitter release (i.e., serving a feedback loop function). This is consistent with the wide distribution of the ECS, both centrally and peripherally, and its involvement in a variety of functions spanning actions from cognition to gestation.

These endocannabinoids interact both with CB_1R and CB_2R, which are G-protein coupled seven-transmembrane receptors. The endocannabinoid system also includes membrane-bound enzymes that catalyze the hydrolytic degradation of the endocannabinoids [i.e., fatty acid amide hydrolase (FAAH), primarily for anandamide and monoacylglycerol lipase (MGL)], for 2-AG, the oxidative enzymes cyclooxygenase-2

(COX-2), and lipoxygenase (LOX), and a putative transport system involved in the reuptake of endocannabinoids [33–35]. Thus, there are several targets for interacting and manipulating the endocannabinoid system(s). Such recent advances in our knowledge about the ECS are backdrops for extending an understanding of the effects of marijuana [36, 37] and, probably, other drugs of abuse [38–41], as well as the role of the ECS in normal, regulatory physiology and in disease states [42].

C. CANNABINOIDS/CANNABINERGICS AND DRUG DISCRIMINATION

The material for this review was primarily obtained through searches using databases provided by PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) and the drug discrimination database available at the Society for Stimulus Properties of Drugs, SSPD (http://www.sspd.org.uk/); the searches were conducted in July and August 2009 (the new website for the SSPD database as of fall 2009: http://www.drugrefs.org/). In the PubMed search, combining "Δ^9-THC and drug discrimination" yielded 119, and combining "cannabinoid and drug discrimination" produced 170 citations. In the drug discrimination database, these key-words produced a similar though slightly higher number of citations. Inclusion criteria for these two databases are different. For example, both book chapters and abstracts are indexed in the drug discrimination data base but not in the PubMed database (see Chapter 3 for further discussion of the two databases). Further subdivision of search terms in the drug discrimination database identified five animal species as being trained with Δ^9-THC—the majority of the studies using rats as subjects (92 being the total number, of which about 58 citations reflect original investigations rather than abstracts and review articles). The four other species identified were birds (pigeons), gerbils, mice, monkeys (chimpanzees and rhesus monkeys), and humans.

D. EXPERIMENTAL PROCEDURES AND SPECIES

1. Rats

Although early drug discrimination studies with Δ^9-THC often utilized maze procedures (e.g., [43]), most of current training and test protocols for infrahuman drug discrimination research are based on operant methodology. Most commonly, animals are trained to discriminate between drug and vehicle in two-choice procedures where the incentive is either appetitive (food or fluid) or aversive (shock escape/avoidance). For example, a food-restricted rat is administered a specified dose of the training drug a preset time before session-start. For rodents, the injection to session onset interval (i.e., PSII; see Chapter 3) mostly has been 20 to 30 minutes after intraperitoneal (i.p.) administration of Δ^9-THC, although the interval has been as long as 60 minutes in some studies [6]. By tradition, lever pressing is commonly used as the operant for rodents. An alternative might be nose poking behavior. This operant manipulandum is increasingly used, particularly in studies with mice [44]. One advantage with nose poking is the phylogenetic,

predisposed nature of the response for rodents. Thus, nose poking requires very little training whereas lever pressing customarily has to be shaped by successive approximations to establish the relationship between the lever response and delivery of reinforcement (see Chapter 3). A variety of reinforcement schedules has been used in drug discrimination research, but fixed-ratio (FR) schedules are very common [45]. As the naming implies, a fixed number of responses (e.g., 10) are required on the state-appropriate lever before reinforcement delivery (FR 10). This reinforcement schedule is also commonly employed for drug discrimination studies using CB_1R ligands as the discriminative stimulus. Drug discrimination studies using Δ^9-THC as the training drug for various strains of out-bred rats have reported discriminative control with doses ranging from 1 mg/kg [46], albeit in a drug versus drug drug discrimination (Δ^9-THC *vs*. psilocybin), to 5.6 mg/kg [47–49], but training doses around 3 mg/kg (3.0–3.2 mg/kg) mostly have been examined.

2. Birds

For pigeons, the operant response has been key-pecking. Commonly, most drug discrimination studies with pigeons have utilized variations of FR schedules of food reinforcement but the FR values usually are set higher than the FR 10 schedule implemented in most operant drug discrimination studies in rats, likely reflecting the ease by which birds associate the pecking response with reinforcement delivery. As is common for rats, the choice manipulandum commonly is space-oriented, that is, pecking a key positioned to the left on the front panel is associated with reinforcement under one training condition (e.g., Δ^9-THC) whereas pecking the key positioned to the right is correct under the alternative training condition (e.g., vehicle or another drug). An alternative would be color tracking where the two keys are trans-illuminated with different colors and these particular colors rather than position (left/right) being associated with drug and vehicle sessions. Such an arrangement allows for changing the position of the state-appropriate key both within sessions and between sessions. Such discriminations have been established with different drugs [50, 51] and have also explored the effects of maintaining drug discrimination responding with reinforcement schedules other than FR schedules [52–55] and time-related events [56]. Using color tracking, drug discrimination stimulus control by four different drug states was established in single subjects [57–59]. Such four-choice drug discrimination requires a very extensive training period and considering also the inclusion of a lengthy test phase, likely are productive only in species with a relatively long life span such as pigeons and monkeys. There is one study [60] involving rats, but no studies to date include cannabinergic ligands as a discriminative stimulus in such elaborate drug discrimination settings. However, Järbe [61] briefly described an unpublished study involving Δ^9-THC, pentobarbital, and vehicle as separate cues in a three-choice procedure (three white trans-illuminated discs positioned to the left, middle, and to the right above the food magazine). It appears that the use of pigeons as experimental subjects is declining both in drug discrimination as well as in behavioral pharmacology research in general but the longevity, in addition to the comparatively high sensitivity to cannabinergics [8] by pigeons, are favorable considerations for this avian species in cannabinoid research. Slow onset and a long duration

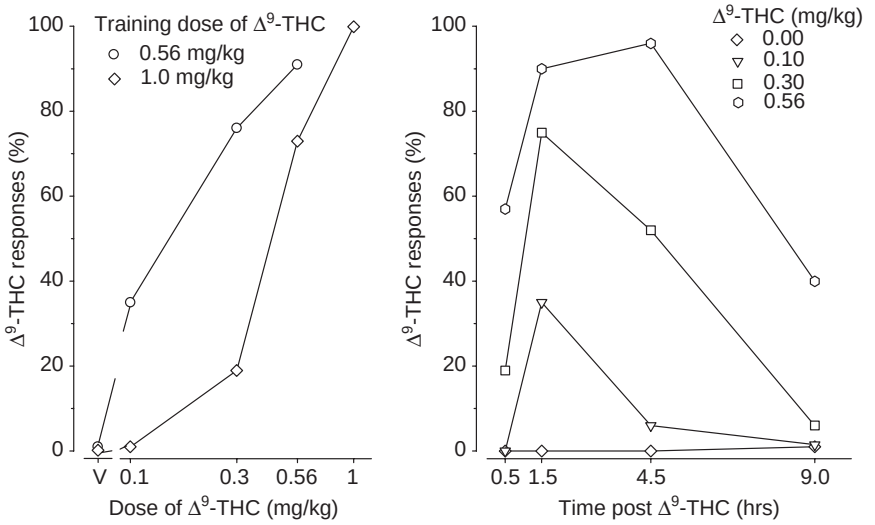


Figure 8-1. Left panel—Δ^9-THC dose-generalization gradients for pigeons trained to discriminate between Δ^9-THC (0.56 mg/kg, n = 7; I mg/kg, n = 8) and vehicle. Training and test sessions were conducted 90 min post-i.m. injection. Data represent the average of at least two determinations for each animal for the 0.56 mg/kg training condition and the average of one determination for the 1.0 mg/kg training condition (note x-axis log scale); the ED_{50} values (± 95% confidence limits) for the dose data (left panel) were: 0.15 (0.11–0.19) and 0.43 (0.39–0.48) mg/kg for the training doses of 0.56 mg/kg and 1 mg/kg, respectively. Right panel—Time course for dose generalization of Δ^9-THC for pigeons trained to discriminate between vehicle and 0.56 mg/kg Δ^9-THC; the ED_{50} values (± 95% confidence limits) for the time-course data (right panel) were: 0.51 (0.50–0.51); 0.15 (0.12–0.19); 0.29 (0.15–0.56); and 0.62 (0.47–0.82) mg/kg for 0.5, 1.5, 4.5 and 9 hr post injection, respectively (nonlinear regression using model: log dose vs. response—variable slope with the top and bottom of the curves constrained to 100 and 0; Prism v. 5). Test sessions utilized a repeated test procedure [63]. Data represent the average of at least two determinations for each animal (n = 7); panels redrawn from [9].

of effect after intramuscular (i.m.) administration characterize Δ^9-THC discrimination in pigeons (Figure 8-1). Comparative studies with rats and pigeons have been performed using Δ^9-THC as the discriminative stimulus and no major species difference in outcome regarding cannabinergic discriminative stimulus control has been reported; enantiomeric selectivity and specificity of the Δ^9-THC molecule in vivo was a focus in several of these studies [62–68]. The training doses of Δ^9-THC have ranged from 0.25 mg/kg [69] to 1.0 mg/kg [68] but 0.56 mg/kg is the dose that has been most commonly used. To the extent studied, the ECS in the avian brain seems similar to that of rodents [70–74] and the uptake and distribution pattern for radioactively-tagged Δ^9-THC also appears similar to that of rodents [75–77]. The effects of Δ^9-THC are specific insofar that Δ^9-THC does not substitute in pigeons trained with amphetamine, cocaine, LSD, morphine, and pentobarbital [78–84], nor does diazepam substitute for the Δ^9-THC cue [85].

3. Monkeys

The initial primate drug discrimination study with Δ^9-THC used chimpanzees as subjects and focused on temporally-related aspects of the discrimination where Δ^9-THC was administered orally in doses ranging between 3 and 4 mg/kg for the individual monkeys [86]; onset and offset of the Δ^9-THC effects were examined using elaborate chained schedules of reinforcement (see Chapter 3). All other primate studies related to cannabinergic drug discrimination stimulus control have used rhesus monkeys whose discrimination behavior was maintained by positive or negative reinforcement. Gold et al. [87] trained rhesus monkeys to discriminate between vehicle and i.m. administered Δ^9-THC where the training dose was individually adjusted and ranged between 0.04 and 0.16 mg/kg. Studies comparing the discriminative stimulus effects of cannabinergics in rats and rhesus monkeys have not reported any major difference in outcome [87–89]. Most Δ^9-THC drug discrimination studies with monkeys employed an i.m. route of administration but, more recently, McMahon and colleagues described an intravenous (i.v.) preparation where stimulus control was maintained by 0.1 mg/kg of Δ^9-THC [90].

4. Mice and Gerbils

There have been two studies using mice and both studies established discriminative stimulus control by i.p. administered Δ^9-THC (10 mg/kg) *versus* vehicle, injected 30 minutes prior to session onset; one study used nose poking as the operant [44], whereas the other study used lever pressing [91]. Both studies used positive reinforcement in food restricted C57BL/6J mice. The one study using gerbils applied different i.p. administered doses of Δ^9-THC to five different groups of animals and examined the acquisition of the drug discrimination as a function of training dose (range 0.5–16 mg/kg) and comparisons were made to a 6th group trained with 20 mg/kg of pentobarbital [92]. As with rats (see below), when trained gerbils (maintained by 2.0 and 8.0 mg/kg of Δ^9-THC) were exposed to hashish smoke, the majority of the gerbils selected the drug/ Δ^9-THC-associated side arm of a T-maze. The incentive for the gerbil study was shock escape.

5. Humans

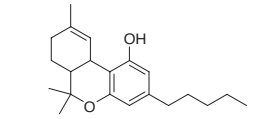
Of the two human studies identified in the literature search, one study used marijuana (2.7% Δ^9-THC) versus placebo (0% Δ^9-THC) smoke [93] as the discriminative cues and the other study used orally delivered Δ^9-THC (25 mg) versus placebo capsules [94]. Money was earned for study participation.

E. TRAINING DRUGS

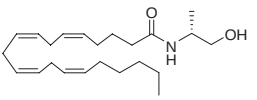
1. Agonists

The vast majority of studies examining the discriminative stimulus effects of cannabinoids/cannabimimetics used the non-selective, mixed CB_1R / CB_2R partial

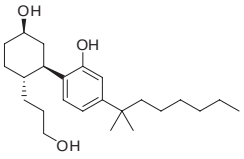
agonist Δ^9-THC as the training drug. Thus, all studies conducted so far with monkeys, pigeons, mice and gerbils (as well as humans) employed the chemically structured tricyclic Δ^9-THC as the drug training condition (see Figure 8-2). In a limited number of reports, the drug training condition has been other cannabinergics such as the potent tricyclic cannabinoid, HU210 [(6a*R*,10a*R*)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen-1-ol] [95], aminoalkylindoles such as WIN55,212-2 [(*R*)-(5-methyl-3-(morpholinomethyl)-2,3-dihydro-[1,4]oxazino[2,3,4-hi]indol-6-yl)(naphthalen-1-yl)methanone] [96], bicyclic cannabinoids such as CP47,497 [2-[(1*R*,3*S*)-3-hydroxycyclohexyl]-5-(2-methyloctan-2-yl)phenol] [97] and CP55,940 [2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl]-5-(2-methyloctan-2-yl)phenol] [98–100], diarylether sulfonylesters BAY38-7271 [(*R*)-3-(2-(hydroxymethyl)-2,3-dihydro-1*H*-inden-4-yloxy)phenyl-4,4,4-trifluorobutane-1-sulfonate] [101, 102], and BAY59-3074 [(-)-(*R*)-3-(2-hydroxymethylindanyl-4-oxy)phenyl-4,4,4-trifluorobutyl-1-sulfonate] [103] as well as anandamide analogs such as AM356 [(*R*,5Z,8Z,11Z,14Z)-N-(1-hydroxypropan-2-yl)icosa-5,8,11,14-tetraenamide; *R*-(+)-methanandamide [49, 104, 105], AM1346 [an alkoxyacid amide of *N*-eicosa-(5Z,8Z,11Z,4Z)-tetraenylamine; structure undisclosed] [106] and O1812 [(*R*,5Z,8Z,11Z,14Z)-20-cyano-N-(1-hydroxypropan-2-yl)-16,16-dimethylicosa-5,8,11,14-tetraenamide] [107]. Some early drug discrimination studies with rats also included the minor, less potent isomer (-)-Δ^8-THC [in early scientific reports sometimes also referred to as (-)-$\Delta^{1(6)}$-THC or (-)-Δ^6-THC) as a training condition [108, 109] in T-maze tasks. Discriminative stimulus effects of Δ^9-THC have also been examined using a discriminated taste aversion (DTA) procedure [110]. Additionally, rats have been trained to discriminate between "placebo smoke" and cannabinoid "active smoke" [3.2% Δ^9-THC, 1.2% cannabinol (CBN; 6,6,9-trimethyl-3-pentylbenzo[c]chromen-1-ol) and 5.1% cannabidiol [CBD; [2-[(1*R*,6*R*)-6-Isopropenyl-3-methylcyclohex-2-en-1-yl]-5-pentylbenzene-1,3-diol)] in a T-maze water escape task [108]. Although the concentrations of cannabinoids resulting from the "active smoke" exposure in the organism were not quantified, the formation of this "smoke"-based drug discrimination developed at a rate comparable to the acquisition rates seen with the training conditions of 5.0 mg/kg Δ^9-THC or 10 mg/kg of Δ^8-THC. Other drug discrimination studies, including Δ^9-THC and Δ^8-THC discriminations [92, 109], have shown that the speed by which drug discriminations are formed is a function of the training dose used such that, within limits, the higher the training dose the faster the drug discrimination is acquired [111, 112]. In addition, other effects of Δ^9-THC (e.g., depression of water intake) were similar to the effects of hashish-smoke exposure [113, 114]. Interestingly, cannabis smoke did not substitute for phencyclidine although phencyclidine delivered as smoke substituted for i.p.-trained phencyclidine drug discrimination, again illustrating the importance of the resulting effect rather than the way by which the effect was produced [115]. Although there is agreement that Δ^9-THC is the major psychotropically active ingredient in marijuana/hashish preparations, it is still debated whether other plant materials contained in cannabis modify the effects of Δ^9-THC. For example, it has been reported that CBD seems to reduce the "anxiogenic" effects of Δ^9-THC in humans [116, 117]. This possibility is one rationale behind Sativex® by GW Pharmaceuticals, an oromucosal preparation



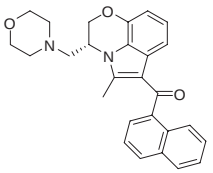
Δ^9-THC
6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol



AM356
(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-((*R*)-1-hydroxypropan-2-yl)icosa-5,8,11,14-tetraenamide



CP55,940
2-((1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl)cyclohexyl)-5-(2-methyloctan-2-yl)phenol



WIN55,212-2
(*R*)-(5-methyl-3-(morpholinomethyl)-2,3-dihydro-[1,4]oxazino[2,3,4-*hi*]indol-6-yl)(naphthalen-1-yl)methanone

Figure 8-2. Chemical structures of some cannabinergics used as training drugs in drug discrimination studies.

combining equal amounts of Δ^9-THC and CBD. Although there is evidence for increased clinical efficacy for the Δ^9-THC /CBD combination [118, 119], other experimental studies have not supported the idea that CBD significantly modifies the psychotropic activity of plant-contained Δ^9-THC at dose ratios commonly encountered in cannabis preparations [120–123]. Given improved techniques for standardized smoke delivery to animals as well as an increased sensitivity of the analytical methods for identifying and quantifying Δ^9-THC and other cannabinoids in body compartments (e.g., [120]), this issue could be revisited using drug discrimination methodology. In humans, rimonabant attenuated but did not completely block the "subjective" effects resulting from cannabis smoke and did not seem to produce any particular "subjective" effects when administered alone [124–126].

2. Antagonists

The first selective CB_1R antagonist, rimonabant (SR141716A; [5-(4-chlorophenyl)-1-(2,4-dichloro-phenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide HCl), was described in 1994 [127, 128], followed by the discovery of additional CB_1R antagonists/inverse agonists such as, for example, AM251 [1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(piperidin-1-yl)-1H-pyrazole-3-carboxamide-*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide], AM281[1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-morpholino-1H-pyrazole-3-carboxamide [129], as well as CB_2R selective antagonists such as SR144528 [5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1*S*,2*S*,4*R*)-1,3,3 trimethylbicyclo[2.2.1]hept-2-yl]-1*H*-pyrazole-3-carboxamide [130] and AM630 [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone] [131]. Figure 8-3 (left) illustrates antagonism of the cueing effects of 1.8 mg/kg Δ^9-THC by AM281 when co-administered 20 min prior to testing. Figure 8-3 (right)

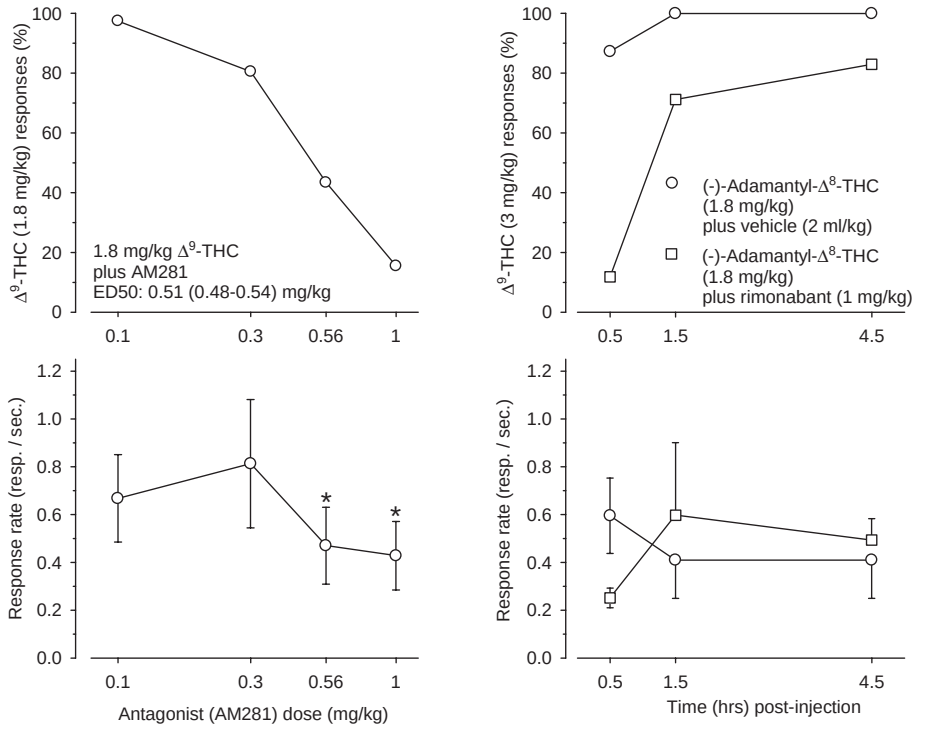


Figure 8-3. Left top panel depicts antagonism of the DS effects of 1.8 mg/kg of Δ^9-THC as a function of increasing doses of the CB_1R antagonist / inverse agonist AM281 (dissolved in 100% DMSO; 1 ml/kg) concomitantly administered i.p. together with Δ^9-THC (n = 8–9), 20 min prior to test session onset. Nonlinear regression (log dose vs. response—variable slope with the top and bottom of the curves constrained to 100 and 0; Prism v. 5) was used to estimate the ED_{50} value (± 95% confidence limits). Lower left panel depicts the response rate associated with testing the AM281 / Δ^9-THC combinations. *($P \leq 0.05$; Holm-Sidak post-hoc comparison procedure involving a control mean = vehicle, applied after significant one-way ANOVA). The average vehicle (5% propylene glycol, 3% tween-80 and saline; 2 ml/kg) control rate (± S.E.M.) for the initial six reinforcements during training sessions initiated 20 min after i.p. injections that immediately preceded these tests was 1.09 (± 0.11) responses per second; the lower and upper 95% C.L. of the control mean being 0.84 and 1.35, respectively. Unpublished data by Järbe and Makriyannis. Right top panel depicts antagonism of the DS effects of 1.8 mg/kg (-)-adamantyl-Δ^8-THC as a function of increasing intervals of the CB_1R antagonist / inverse agonist rimonabant administered i.p. together with 1.8 mg/kg (-)-adamantyl-Δ^8-THC (n = 8) 30 min prior to first test session onset in rats trained to discriminate between 3 mg/kg Δ^9-THC and vehicle. Lower right panel depicts the response rate associated with testing the rimonabant / (-)-adamantyl-Δ^8-THC combinations. The graphs to the right were adapted from data originally described by Lu et al. [221]. A repeated tests procedure [63, 239] was used to assess the time course of (-)-adamantyl-Δ^8-THC and its combination with rimonabant. Thus, rats were injected i.p. with (-)-adamantyl-Δ^8-THC and first put into the experimental chamber 30 min post-administration. The second test took place 90 min post, and the third (final) test occurred 270 min after administration. Between the test trials, the animals waited in their respective home cages. Doses were examined in a mixed order. Further details in Lu et al. [221].

illustrates antagonism of the cueing effects of the CB_1R agonist AM411 [6a*R*,10a*R*)-3-(1-adamantyl)-6,6,9-trimethyl-6a,7,10,10a-tetrahydrobenzo[*c*]chromen-1-ol; (-)-adamantyl-Δ^8-THC) by rimonabant at different intervals after administration of the drug combination.

To date, there are three reports attempting to establish rimonabant as a discriminative stimulus in monkeys, rats and pigeons and all three studies were unsuccessful in achieving that goal using operant methodology [96, 132, 133]. However, Järbe et al. [110] reported the successful implementation of establishing rimonabant as a discriminative cue in rats using taste aversion-based methodology (discriminated taste aversion; DTA). The acquisition of the rimonabant (5.6 mg/kg) drug discrimination was surprisingly rapid given the failures described above using operant methodology. The acquisition data from the first such study are depicted in Figure 8-4A. The use of 5.6 mg/kg of rimonabant was accompanied also by signs of unconditioned effects as determined

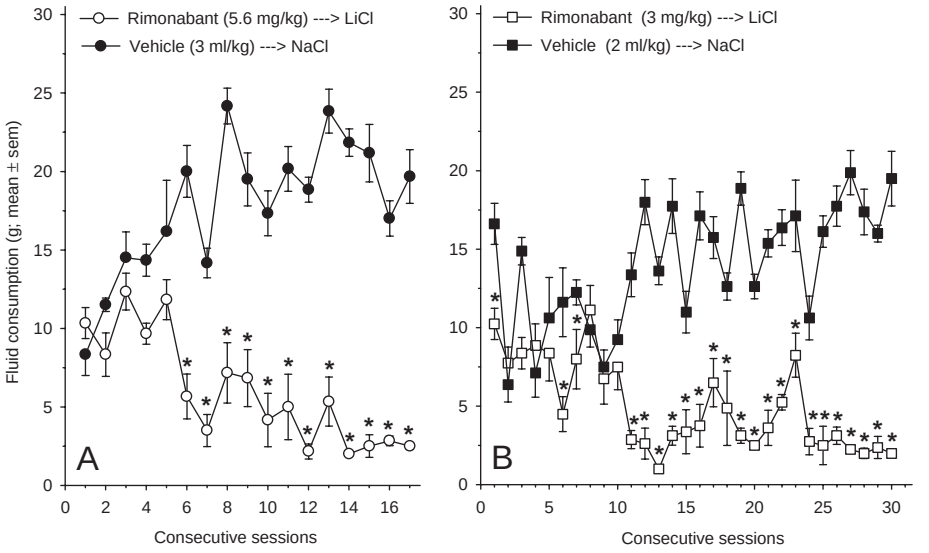


Figure 8-4. Panel A depicts acquisition of rimonabant (5.6 mg/kg) discrimination in rats using a taste aversion approach (discriminated taste aversion; DTA) and panel B depicts acquisition of a similar DTA using a lower dose of rimonabant (3 mg/kg) from the outset of training. Y-axes: average (± S.E.M.) fluid consumption by six (panel A) and eight (panel B) rats, respectively, during a 30 min fluid offering. X-axes: consecutive sessions alternating between rimonabant (open symbols) and vehicle (filled symbols) initiated 20 min after i.p. pretreatment. Drinking under the influence of rimonabant was followed by i.p. injections of 120 mg/ml (10 ml/kg) lithium chloride (LiCl), occurring immediately after the 30 min drinking bout. Drinking after vehicle was followed by 10 ml/kg sodium chloride (NaCl). Additional water access of 30 min duration occurred in the afternoons. *($P \leq 0.05$; Holm-Sidak post-hoc pairwise comparison procedure) signifies statistically verified differences in fluid consumption between adjacent rimonabant and vehicle sessions. Panel A adapted on data published in [110] and panel B is based on data originally presented in [134].

in a separate control group (not shown). These initial data were replicated in a second study [134], which also aimed at exploring the potential use of lower maintenance doses of rimonabant. Rats initially trained to discriminate between vehicle and 5.6 mg/kg of rimonabant subsequently were retrained with 3.0 mg/kg of rimonabant. This "new" discrimination was acquired rapidly and dose-tests with rimonabant verified the dose-dependent nature of the discrimination. After completing dose generalization tests, the training dose of rimonabant was lowered further to 1.8 mg/kg. Discriminative control of behavior by 1.8 mg/kg of rimonabant was statistically verified but it is unclear if the degree or magnitude of the resulting control over fluid consumption would serve as a useful baseline for extended training and testing. Subsequently, a new group of rats was trained to discriminate between 3.0 mg/kg of rimonabant and vehicle from the outset of the study. The acquisition data are shown in Figure 8-4B and a comparison between the two sets of acquisition data suggests that more training sessions were needed for the low-dose condition to achieve discriminative control at a level comparable to that of the high-dose condition. The difference in fluid intake between drug and vehicle sessions for the controls corresponding to the 3.0 mg/kg dose of rimonabant discrimination dose were attenuated compared to the controls accompanying the 5.6 mg/kg of rimonabant training dose, signifying reduced unconditioned effects for the 3.0 mg/kg of rimonabant discrimination (not shown). McMahon [132] reported readily acquired stimulus control by rimonabant in monkeys pretreated with Δ^9-THC, although as noted above, rimonabant alone failed to control the choice behavior of monkeys examined under similar experimental conditions. Such outcome patterns have been observed also with, for example, opioid ligands in the drug discrimination literature. Thus, opioid antagonists such as naloxone and naltrexone are difficult to establish as discriminative cues in drug-naïve subjects but serve much more readily as a cueing function in animals pretreated with opioid agonists using operant methodology [135–139]. Yet, naloxone alone readily served as a cue for rats using DTA methodology and displayed orderly dose- and time-dependent effects that appear centrally mediated and the naloxone cue was pharmacologically selective [140–142]. Altered sensitivity by pharmacological and/or other means might be useful avenues for cannabinergic drug discrimination's that perhaps can capture drug discrimination functions of, for example, CB_2R ligands that likely would be difficult to establish as discriminative cues in "normal" subjects. For example, administration of aspirin more clearly served a discriminative function for "arthritic" as opposed to "normal," nonarthritic rats [143]. Additionally, the discriminative stimulus produced by clonidine in spontaneously hypertensive rats resulted in generalization to antihypertensive drugs with different mechanisms of action. Given the convergence in results across different receptor mechanisms, it was concluded that these discrimination and generalization results likely were based on shared antihypertensive actions [144]. Along similar avenues, it was recently reported that for rats discriminating between 22- and 2-hour food restriction, the appetite suppressant drug sibutramine, but not rimonabant, shifted responding from the lever associated with 22-hour food restriction to the lever associated with the shorter restriction interval [145]. It was suggested that sibutramine produced this lever-selection shift because of reduced hunger-sensation, whereas the basis for rimonabant-produced suppression remains to be elucidated (see also [146]).

F. PROCEDURAL CONSIDERATIONS

1. Route of Administration

As is the case for most drug discrimination research, the i.p. route of administration is nearly universally employed for training and testing cannabinergics in rodents. Rats trained to discriminate between i.p. 2.0 mg/kg Δ^9-THC and vehicle were also examined after i.v. and oral application and as expected, onset and offset as well as the potency of the discriminative stimulus effects of Δ^9-THC co-varied with the route of administration. Thus, Δ^9-THC administered i.v. resulted in the fastest onset and offset and the lowest median dose effect estimate value (ED_{50}), suggesting increased potency, followed by the i.p. and oral routes of administrations [147]. A few studies used intraventricular (i.v.t.) administration or in situ brain injection to examine site of action of cannabinoids [148, 149]. In monkeys and pigeons the i.m. route has been most commonly employed; recently, McMahon [90, 132] described an i.v. preparation for drug delivery to rhesus monkeys being trained to discriminate between CB_1R agonists and antagonists and the vehicle condition in a two-choice procedure. As noted above, the two human drug discrimination studies used either marijuana-smoke delivery or Δ^9-THC delivered orally [93, 94].

2. Vehicle(s) for Cannabinergic Drugs

A note of caution concerns the added complexity resulting from the lipophilic character of cannabinergic ligands when comparing results across different laboratories. Hence, different vehicles are used in different laboratories to prepare suspensions and may differ in their efficiency as carriers of these molecules. Unfortunately, there are no recent systematic comparisons of the more common currently used vehicles for cannabinoid compounds. This could very well be the reason why the onset by rimonabant, suspended in only 0.3% Tween-80, in antagonizing the discriminative stimulus effects of Δ^9-THC appeared slow in a study by Solinas et al. [150] compared to, for example, Järbe et al. [49, 104], where rimonabant first was dissolved in 3–4% Tween-80 and 5% propylene glycol before slowly adding saline to the final volume of 2.0 or 3.0 ml/kg depending on the concentration of rimonabant. Water-soluble cannabinergics have been reported [151, 152], but demonstration that the uptake and distribution pattern(s) of these salts are the same as those for lipophilic cannabinergics will require additional examination. For example, the antinociceptive effects of intrathecally administered cannabinoids seem influenced by their lipophilicity [153]. One such water-soluble analog (Org 28611; structure undisclosed) has been examined in humans and the "subjective" effects appeared similar to those described for Δ^9-THC but clearly different from the benzodiazepine midazolam [154]. An ester of Δ^9-THC [SP-111; (-)-(6a*R*,10a*R*)-6,6,9-trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-yl-4-morpholinobutanoate] was solubilized in saline alone and its stimulus effects examined in rats and pigeons discriminating between Δ^9-THC and vehicle [Tween 80 (4%) / propylene glycol (5%) / saline] and the ester resulted in a delayed onset of effect, particularly for pigeons [68]; see also [155].

G. INTENDED AND UNINTENDED "BIAS" IN DRUG DISCRIMINATION

Experimental manipulations of reward density [55, 156–158] can markedly influence the outcome of drug discrimination studies, such that, generally, the lower the reinforcement density experienced during a particular training condition, the more likely the animal is to choose the alternative response. For example, when more effort is required by the subject to produce a reward in nondrug training as compared to the drugged sessions, the dose-generalization gradients flattened. When the requirements were reversed, the slope of the gradient became steeper. The experimentally manipulated "bias" was also reflected in tests of antagonism and in the degree of generalization to other drugs.

Mokler et al. [159] reported that 13 out of 20 rats trained to discriminate 3.0 mg/kg of Δ^9-THC from vehicle evoked close to 100% drug (i.e., Δ^9-THC)- appropriate responding in tests with the benzodiazepine diazepam. This, no doubt, is an interesting finding indicating that Δ^9-THC may exhibit "anxiolytic" activity via benzodiazepine receptors; see also [160]. However, this conclusion seems compromised by the percentage of correct-responding in the maintenance (i.e., training) sessions being 99% for drug- (D) and 88% for nondrug (N) maintenance sessions, respectively, indicating possible bias to the drug (Δ^9-THC)-associated lever/position. Since not all rats displayed generalization in tests with diazepam, this study points toward individual sensitivity/variability as a factor also influencing drug discrimination results (for further discussion see [61, 161]). Suffice here is to note that one study [162] suggested that individual sensitivity to the effects of Δ^9-THC revealed itself by such rats acquiring the original Δ^9-THC (3 mg/kg) discrimination faster than less sensitive rats and also by exhibiting a lower ED_{50} value of 0.77 mg/kg, whereas the ED_{50} for the less sensitive group was 1.63 mg/kg.

H. ORIGIN OF THE DRUG STIMULUS AND SENSORY MEDIATION

These issues were discussed by Järbe [61, 161], and the interested reader is referred to these publications for more detail. It was concluded that central action is not a prerequisite for drug discrimination (see Chapter 3), but if the reference (i.e., training-drug dose) was primarily centrally acting, the contribution of peripheral effects in controlling the discrimination generally appeared negligible (e.g., see Chapter 3). Few studies have reported success with training peripherally-constrained drugs except in a select few cases (e.g., the muscarinic blocker isopropamide [163]). Four of the initial six rats reached the discrimination criterion and subsequently demonstrated an orderly reduction in the number of subjects selecting the drug-associated lever response in tests with progressively lower doses of the training compound; the peripherally restricted muscarinic anticholinergic agent methylscopolamine substituted for isopropamide. Methylscopolamine does not substitute in animals trained with a centrally acting anticholinergic drug [164]. Additionally, rats discriminating between a low dose (0.16 mg/kg) of racemic amphetamine and saline generalized to the peripherally constrained para-hydroxyamphetamine but not to a high dose (5 mg/kg) of (*±*)-amphetamine [165],

suggesting a possible qualitative difference between peripherally and centrally mediated drug cues [166]. Likely, the discriminative stimulus effects of cannabinergics examined in the existing literature all were centrally mediated even though this assumption has not been directly verified pharmacologically in all but one case. Pério et al. [96] showed dose-dependent antagonism by rimonabant of the training drug WIN55,212-2 as well as the generalization to WIN55,212-2 by CP55,940 and Δ^9-THC, whereas no antagonism of WIN55,212-2 was observed after co-administration of the rimonabant analog SR140098 (structure undisclosed), a purportedly peripherally restricted CB_1R antagonist, suggesting central mediation for the discriminative stimulus effects of the CB_1R agonist(s). Attempts have been made to characterize specific brain sites that might underlie the discriminative stimulus effects of Δ^9-THC. Mokler and Rosecrans [149] applied intracranial administration of Δ^9-THC to the prefrontal cortex, dorsal hippocampus, and reticular formation but these efforts were met with limited success. However, i.v.t. administration of Δ^9-THC substituted for systemic Δ^9-THC, as described in an abstract, even though it is unclear which brain area(s) were responsible for mediating the effect [148]. Following the observation that Δ^9-THC can promote increased extracellular levels of the endogenous opioid β-endorphin in the ventral tegmental area, rats discriminating between Δ^9-THC (3.0 mg/kg) and vehicle exhibited a leftward shift of the generalization curve when tested with subthreshold doses of Δ^9-THC in combination with *in situ* applied β-endorphin. Parallel studies with morphine showed similar augmentation of Δ^9-THC dose-generalization and the effects of both opioids were blocked by the opioid antagonist naloxone. Neither of the two opioid agonists substituted for Δ^9-THC when administered alone [167]. This anatomical site for "cross-talk" between the ECS and endorphins overlaps with systems or neuronal circuitries implicated for Δ^9-THC induced locomotor activation, intracranial self-administration and other reinforcement processes such as those measured by conditioned place preference [168].

I. ACQUIRED DIFFERENCES IN DRUG SENSITIVITY

1. Drug Discrimination and Tolerance

Repeated administration of a drug may lessen the effect(s) such that an escalation in dosing is necessary to obtain the desired effect or the initially observed response to the drug in question. This loss in efficacy is commonly referred to as development of tolerance. A highly controversial issue in drug discrimination research is whether or not the discriminative stimulus properties are subject to development of tolerance [169].

Three approaches generally have been tried. One has been to pretreat animals with the proposed training drug for a certain period of time and then compare the rate of acquisition of the drug discrimination of pretreated and non-pretreated animals. The basic assumption is that acquisition is related to dose such that the higher the dose used in training, the more rapid the formation of the discrimination. Thus, tolerance is expected to retard acquisition of drug discrimination. Given this dependent variable, such approach has not indicated tolerance employing a variety of drugs [170–173].

Concerning cannabinoids, Bueno and Carlini [174] observed tolerance to behavior patterns initially disrupted by the administration of a marijuana extract. Despite the fact that seemingly complete tolerance developed to rope climbing, subsequent employment of a drug discrimination task showed that the drug still induced measurable effects. Unfortunately, this study did not include animals without pretreatment. Therefore, it is not possible to evaluate effects on the speed of acquisition of the drug discrimination task by pre-exposure to Δ^9-THC. Järbe and Henriksson [175] included animals that were not pretreated with drug and concluded that prior exposure to Δ^9-THC did have an effect on the subsequent establishment of the Δ^9-THC discrimination. However, subsequent data by Järbe (unpublished) that utilized several pretreatment doses of Δ^9-THC indicated that tolerance effects to the Δ^9-THC stimulus are small in magnitude when using pre-exposure to ascertain tolerance.

Another approach has been to examine the dose-generalization gradients repeatedly over a long period of time [170, 176, 177], but no significant changes in the ED_{50} values of the opioid agonist fentanyl and the motor stimulant cocaine occurred after extended training and testing. This has been the case also when examining cannabinergis [63, 178, 179]. However, the inherent problem when evaluating tolerance development this way is that its occurrence might be masked by the animals learning a progressively more difficult task. In this regard, it is important to keep in mind that the generalization gradient and hence the ED_{50} dose generally is related to the training dose (Figure 8-5); see also [109 and Chapter 3].

A third approach involves the administration of comparatively high doses of the training drug during a period when training is suspended. In many instances, this latter approach has indeed indicated tolerance development to the stimulus effects of, for example, morphine, amphetamine, cocaine and nicotine [180–189]. Concerning Δ^9-THC, the outcome has varied. Hirschhorn and Rosecrans [190] obtained mixed results depending on the dosage used. However, administration of high or noncontingent doses of this compound did alter the discriminative stimulus effects of Δ^9-THC. When animals trained to discriminate Δ^9-THC from nondrug and then administered high doses of Δ^9-THC or analogs, tests conducted 24 to 48 h after high-dose administration resulted in an attenuation of the Δ^9-THC cue [191, 192]. The relative potencies for Δ^9-THC and an exocyclic analog $\Delta^{9(11)}$-THC have varied [88]. The most pronounced demonstration of tolerance development to the discriminative stimulus effects of Δ^9-THC using suspended training along with supplemental Δ^9-THC administration was by Wiley et al. [193] in a two-lever choice procedure for rats. The investigators observed a 40-fold shift to the right of the Δ^9-THC generalization curve and a return of the curve to the original position after 23 days after terminating the supplemental Δ^9-THC dosing. Nonetheless, noncontingent exposure apparently is not sufficient to disconnect the future association between the drug stimulus and the behavioral consequences.

2. Drug Discrimination and Withdrawal (“Physical Dependence”)

Rats and pigeons were made tolerant to morphine and subsequently were trained to discriminate between this opioid-tolerant, “normal” state and the effects caused by

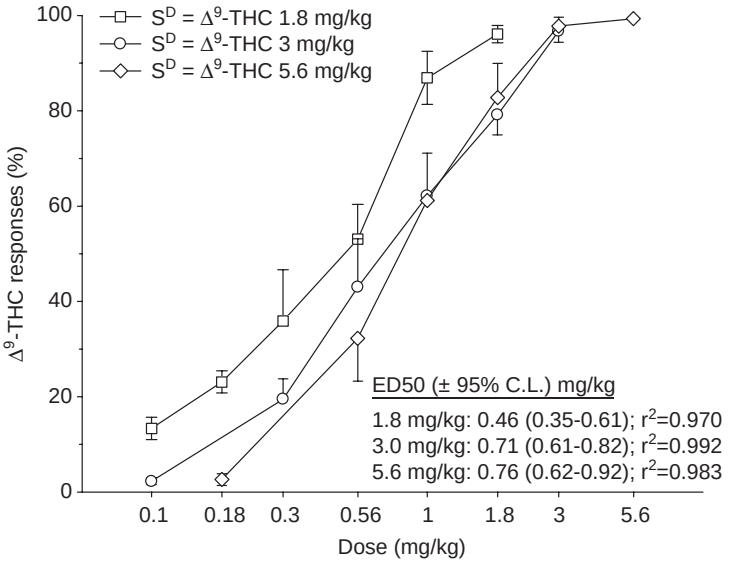


Figure 8-5. The plots summarize dose-generalization gradients (mean ± S.E.M.) as a function of different training doses of Δ^9-THC from several drug discrimination studies (both published and unpublished) using an operant two-lever choice procedure (FR 10) for food restricted male Sprague-Dawley rats (Taconic Farms, NY); S.E.M. reflects variability across studies for a particular dose examined. All injections were i.p. occurring 20 or 30 min prior to training or test session onset. Training sessions were of 20 min duration and test sessions terminated after six reinforcements or 20 min had elapsed, whichever occurred first. There was one session per test day. Doses and drugs were examined in a mixed order. For each dose tested, the percentage of responding on the drug-appropriate lever was calculated from the ratio of the number of presses on the drug (Δ^9-THC) associated lever to the total number of lever presses in a test session. Only data for animals receiving at least one reinforcer during the test session were considered for this measure, that is, animals must have made a minimum of 10 presses on one of the two levers. Test (T) sessions were conducted on average 3 times every two weeks; on interim days, regular drug (D)- or vehicle (V) training sessions of 20 min. duration took place. Typically, the order of sessions was: D, V, T, V, D (week 1); V, T, V, D, T (week 2); V, D, T, D, V (week 3); and D, T, D, V, T (week 4). Tests were conducted only if responding during the preceding training sessions had been correct (FRF ≤ 14) during the initial six FR 10 cycles of the session. If incorrect, animals were retrained for at least three sessions where FRF ≤ 14 before additional testing took place. The curves for 1.8 and 3 mg/kg are based on 5 to 8 independent studies with n's ranging from 8 to 12 and the curve for 5.6 mg/kg is based on 3 independent studies (n's ranged from 7 to 8). All data points were included for the nonlinear regressions [equation: log dose vs. response—variable slope with the top and bottom of the curves constrained to 100 and 0] using the software program Prism (v. 5). For comparison, linear regressions (with log transformation of dose; Prism v. 5) involving all doses except 5.6 mg/kg resulted in the following ED_{50} (± 95% C.L.) estimates (mg/kg): 1.8 mg/kg; 0.40 (0.31–0.52); 3 mg/kg; 0.62 (0.48–1.02); and 5.6 mg/kg; 0.76 (0.62–0.93). When excluding the lowest test doses (i.e., 0.1 and 0.18 mg/kg) for the two higher training doses of Δ^9-THC from the linear regressions, the estimates were 0.77 (0.56–1.01) m/kg and 0.82 (0.56–1.04) mg/kg for the 3 and 5.6 mg/kg training conditions, respectively.

administration of opioid antagonists [138, 139, 194]. Presumably the state of morphine withdrawal distress served as the discriminative event in these studies (see also [195]). Altered sensitivity to the antagonist was indicated by the very low doses of naltrexone needed to establish the discrimination in comparison to the doses required in narcotic-free animals; the results from generalization tests also differ [135, 138, 194]. Both "spontaneous" and antagonist-precipitated withdrawal has been reported for diazepam-tolerant rats in a drug discrimination procedure [196, 197]. "Spontaneous" withdrawal symptoms have been described after discontinuation of Δ^9-THC administration in monkeys [198] and man [199] and more robustly after CB_1R antagonist (rimonabant) precipitated termination of Δ^9-THC adaptation in rodents and dogs [summarized in [200, 201]]. A similar antagonist challenge in rats that were continuously infused with anandamide did not result in overt withdrawal signs [202]. Data by McMahon and colleagues [132, 203] point towards further analysis of Δ^9-THC withdrawal symptomatology using drug discrimination based methodology. There is clearly a need for pharmacological intervention(s) in the management and treatment of cannabis dependence [204, 205].

J. PHARMACOLOGICAL SPECIFICITY

The two most commonly used screens for detecting centrally acting CB_1R ligands are the tetrad (locomotor activity, hypothermia, antinociception, and catalepsy) and drug discrimination. Drug discrimination is considered the more pharmacologically selective of the two but is also more labor intensive. The most comprehensive examination of the specificity of the Δ^9-THC cue under uniform conditions was presented by Browne and Weissman [6]. Of the 30 non-cannabinoid compounds examined, all but two yielded less than 40% Δ^9-THC appropriate responding. The two exceptions were the anesthetic althesin and the benzodiazepine diazepam, resulting in 60% and 52% Δ^9-THC appropriate responding, respectively. Balster and Prescott [2] cautioned that even though the results presented by Browne and Weissman were indicative of very good pharmacological specificity, representatives from several drug classes had not been included in the test protocol. However, additional tests conducted both before and after these results were published continue to suggest that the Δ^9-THC cue is very specific across species, including man [44, 91, 94, 132, 206, 207]. For example, mice trained to discriminate between vehicle and i.p. administered Δ^9-THC (10 mg/kg) responded in a manner indicative of absence of the discriminative stimulus effects of the training drug when tested with nicotine [91], alcohol, ketamine, and cocaine [44], even at behaviorally disruptive doses. In humans, pharmacological specificity was demonstrated with triazolam, hydromorphone, and methylphenidate [94]. The pharmacological selectivity seems to extend also to situations where other CB_1R ligands or doses have been used as the cues for drug discrimination training [48, 103, 105]. It is nonetheless interesting to note that a relatively high degree of Δ^9-THC -like responding has continued to resurface when testing diazepam in rats and monkeys [206, 207], but not in animals trained with the full CB_1R agonist CP55,940 [99]. The partial generalization

elicited by diazepam was blocked by the benzodiazepine antagonist flumazenil but not by rimonabant in rats [208]. However, intragastrically administered diazepam (5.6–30 mg/kg) did not produce much Δ^9-THC -like responding in pigeons discriminating between vehicle and 0.56 mg/kg of Δ^9-THC irrespective of the several post-administration tests examined. Additionally, in gerbils trained with benzodiazepine agonists (5.6 mg/kg diazepam or RO11-3128), Δ^9-THC doses of 5.6 and 17.5 mg/kg did not evoke benzodiazepine-like responding [85]. Given that the degree of specificity likely is co-determined by the training dose used [209], it is prudent to examine this issue whenever a new dose is used as the training condition. For rats trained with 1.8 mg/kg of Δ^9-THC or 10 mg/kg of AM356, very limited drug appropriate responding occurred in tests with either amphetamine or morphine [48, 105]. However, for rats trained with 3.0 mg/kg of the high-affinity CB_1R selective AEA analog AM1346, tests with 3.0 mg/kg of amphetamine resulted in ≈50% drug appropriate responding but much less drug-like responding after these rats had been retrained with a higher dose of AM1346 (5.6 mg/kg). On the other hand, tests with morphine resulted in only a very limited degree of AM1346-like responding irrespective of the training dose of AM1346 [106]. Pharmacological specificity was evident also for the rimonabant discriminations using DTA in that naloxone, flumazenil, amphetamine, morphine, AM356 and Δ^9-THC failed to suppress drinking the way conditioning with rimonabant controlled drinking [110, 134]. Δ^9-THC (1.8 mg/kg) drug discrimination based on DTA also was pharmacologically selective and Δ^9-THC did not substitute for DTA based morphine drug discrimination [110, 210].

Having noticed that the discriminative stimulus effects of cannabinergics are unique in that only other cannabinergics substituted for Δ^9-THC [2], more recent work has focused on delineating mechanism(s) of action. Already from previous reviews it was clear that cannabinergic-like compounds exhibited strict structural requirements in eliciting Δ^9-THC -like activity. By restricting themselves to one species (rat) and two-lever operant choice procedures, made it possible for Balster and Prescott [2] to calculate the range of relative potencies which was found to span between 50 below to 100 times above the potency of Δ^9-THC in eliciting Δ^9-THC -like cue effects. Further, Compton et al. [211] found reasonably high correlations between binding affinity for CB_1R and in vivo potency in both the rat drug discrimination model ($r = 0.81$) and for "psychotomimetic" activity in humans ($r = 0.88$) as well as for the measures comprising the tetrad test battery. Excluding AEA and related analogs (see later), additional CB_1R agonists subsequently appearing in the literature do not seem to contradict these initial correlations between CB_1R affinity and Δ^9-THC-like discriminative stimulus effects in rats, although both ligand specific CB_1R activation (by engaging, e.g., different G proteins) and non-CB_1R mechanisms have been implicated [66, 87–89, 99, 192, 212–221]. Of course, peripherally constrained CB_1R agonists would represent "false" negatives as would antagonists. Theoretically, to the extent of being a partial as opposed to a full agonist, Δ^9-THC conceivably could also act by blocking activity resulting from the release of endocannabinoids, particularly 2-AG [222]; see also [223]. Yet, demonstration of potential partial Δ^9-THC or AM356 agonism using cannabinergic drug discrimination is very limited [44, 98].

K. PHYTOCANNABINOIDS AND METABOLITES

1. Cannabidiol (CBD) and Cannabinol (CBN)

More than 60 cannabinoids (C_{21} terpenophenolic cannabinoids) have been identified from cannabis preparations [224]. All in all, more than 525 constituents have been identified from *Cannabis sativa* L. The most commonly examined cannabinoids have been Δ^9-THC, CBD, and CBN. Although CBD does not bind or binds only weakly to the CB_1R and is non-cannabimetic in humans [225, 226], a renewed interest in this phytocannabinoid has been spurred by reports on potential anxiolytic and antipsychotic actions [227–230]. As discussed earlier, there is only limited evidence to suggest that CBD significantly affects the "subjective high" at CBD concentrations commonly encountered in cannabis preparations consumed for recreational purposes. CBD neither generalizes to, nor does it block, the discriminative stimulus effects of Δ^9-THC either in mice, rats, or pigeons [62, 69, 231–233], the exception being a T-maze study where co-administration of CBD (40 mg/kg) and Δ^9-THC (1.25 and 5 mg/kg) attenuated Δ^9-THC-like responding [234]; CBD by itself did not generalize to Δ^9-THC in the latter study. Although not blocking the discriminative stimulus effects of Δ^9-THC (3.0 mg/kg) in rats, in the same report Vann et al. [233] found that 1.0 and 10 mg/kg of CBD blocked conditioned place aversion induced by 10 mg/kg of Δ^9-THC in mice. Such preclinical data seem supportive of the rationale for the combination of CBD / Δ^9-THC in Sativex®. At even higher dose ratios, CBD (30 mg/kg) may prolong the action of Δ^9-THC (1.0 mg/kg) in rats, possibly by competing for the liver enzymes responsible for the metabolism of these cannabinoids [235]; such prolongation of the Δ^9-THC discriminative stimulus effects was not observed in pigeons [62]. Additionally, structurally related CBD analogs did not substitute for Δ^9-THC either in pigeons or rats [63, 232].

Another phytocannabinoid that is receiving renewed attention and has been examined in drug discrimination is CBN. Most authors report that the discriminative stimulus effects of the CB_1R partial agonist CBN substitutes for Δ^9-THC [6, 7, 65, 99, 231, 236, 237], albeit with lesser potency compared to Δ^9-THC. CBN is a less potent cannabimetic than Δ^9-THC also in humans [225], and its activity in man may depend on the route of administration [226, 238]. The time-course for the Δ^9-THC-like discriminative stimulus effects of CBN was very similar to that of Δ^9-THC both in rats [63, 239] and pigeons [62]. Thus, maximum effect was seen at 30 minutes (rats) and 90 minutes (pigeons) after i.p. and i.m. administration in the two species, respectively, followed by a gradual decline in Δ^9-THC-like responding and by the last daily test probe at 4.5 (rats) and 9 hours (pigeons) after the initial CBN application, responding predominantly occurred on the lever/key associated with vehicle rather than the Δ^9-THC training condition. Co-administration of CBN and Δ^9-THC resulted in additive effects when evaluated in animals trained to discriminate between 3 mg/kg (rats) and 0.56 mg/kg (pigeons) of Δ^9-THC and the vehicle condition. The additivity in Δ^9-THC-like effect by co-administration of less than maximally effective doses of CBN and Δ^9-THC appeared more pronounced for rats than pigeons [65], but this differential effect may depend more on the route of administration rather than reflecting a true species difference.

For humans, Hollister [240] reported that co-administration of CBN and Δ^9-THC prolonged the cannabimetic action. Additive effects on some measures were reported by Karniol et al. [241] when testing humans with combinations of CBN and Δ^9-THC. However, although sharing overlapping pharmacological effect spectra, the cannabimetic activity of Δ^9-THC and CBN may not be identical. Humans self-administering the drugs i.v. reported feeling less anxious after CBN compared to Δ^9-THC infusions, perhaps reflecting differences in intrinsic activity between the two partial agonists. No discernable psychological effects occurred after i.v. infusions delivering CBD in the study [226]. Unlike the discriminative stimulus effects of combinations of Δ^9-THC and CBD described above, the Δ^9-THC-like DS effects of CBN in rats were attenuated slightly with CBD (10 and 30 mg/kg) / CBN (10 and 17.5 mg/kg) combinations [242]. Yet, temperature and open-field activity in rats were not altered by such combinations [243], whereas temperature and open-field recordings in rats after i.p. CBN administration alone [65] produced an effect spectrum similar to that observed after Δ^9-THC treatment [245–247]. Using the acetic acid stretching test, a rodent pain model, Booker et al. [248] recently observed analgesia with both Δ^9-THC and CBN at doses lower than those required for suppression of locomotor activity. Another phytocannabinoid tetrahydrocannabivarin (THCV; 6,6,9-trimethyl-3-propyl-6a,7,8,10a-tetrahydro-6*H*-benzo[c]chromen-1-ol) antagonized the analgesia produced by Δ^9-THC and CBN (for review on the complex pharmacology of phytocannabinoids, see Pertwee [16]). No studies have examined the discriminative stimulus effects of THCV and plant materials other than CBD and CBN in a systematic fashion. Analogs/derivatives of CBN have been described [249, 250] and side-chain branched derivatives such as 11-OH-CBN-DMH showed the highest affinities for cannabinoid receptors as is also the case for corresponding THC based derivatives such as HU210 and the structurally related hexahydrocannabinol derivative HU243 [(6a*R*,10a*R*)-9-(hydroxymethyl)-6,6-dimethyl-3-(2-methyloctan-2-yl)-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-1-ol], exhibiting potent in vivo cannabimetic activity in the drug (Δ^9-THC) discrimination model [67, 251, 252]. Such CBN derived molecules have not been tested in the cannabinergic drug discrimination model.

2. Cannabinoid Metabolites

The metabolism of Δ^9-THC is complex and varies between species [225]. Metabolites that have been isolated and examined with Δ^9-THC-trained animals include 11-hydroxy-THC (both Δ^8- and Δ^9-11-OH-THC). Collectively, the data indicate that 11-OH- Δ^9-THC is a major psychotropic metabolite exerting Δ^9-THC-like activity of biological significance. Investigations with animals trained to discriminate between Δ^9-THC and the no-drug state indicate that the effects of the 11-hydroxylated forms of Δ^8- and Δ^9-THC are similar to the Δ^9-THC stimulus. The cannabimetic activity of the 11-hydroxy metabolites of Δ^8- and Δ^9-THC has been investigated using rats [6, 7, 68, 147, 232] and pigeons [68, 244]. Both species generalized from the Δ^9-THC stimulus to the test compounds in a dose-related manner. Järbe and McMillan [68] found 11-OH-Δ^9-THC to be more potent than 11-OH-Δ^8-THC and that 11-OH-Δ^8-THC was at least as potent as Δ^9-THC whereas Ford et al. [232] found also the 11-OH-metabolite

of Δ^8-THC to be more potent than the Δ^9-THC training stimulus. Whatever the reason(s) for this difference, the potency estimates derived from Δ^9-THC discriminating animals are similar to those reported for humans [240, 253, 254]. Other Δ^9-THC metabolites that have been studied for their cannabimetic effect are 8α-OH-Δ^9-THC, 8β-Δ^9-THC, 8α,11 di-OH-Δ^9-THC, and 8β,11 di-OH-Δ^9-THC [68, 232]. Only the latter compound, 8β,11 di-OH-Δ^9-THC, produced complete generalization to the Δ^9-THC stimulus by pigeons. The metabolite 8β-OH-Δ^9-THC did produce partial generalization (≈40%) for the Δ^9-THC stimulus [68]. Other investigations utilized rats and did not find generalization for the Δ^9-THC stimulus with these metabolites though some generalization (≈51%) occurred with 8β-OH-Δ^9-THC [232]. However, because lower doses of the test compounds were used for the tests with rats and pigeons tend to be more sensitive than rats to the Δ^9-THC cue, further tests using higher doses of these metabolites with rats must be conducted before a conclusion about species differences can be drawn. In addition, the two epimers of the 8-0H metabolite of Δ^9-THC have been examined in humans. Perez-Reyes et al. [255] concluded that only 8β-OH-Δ^9-THC was weakly Δ^9-THC-Iike; whereas Hollister [240] reported that both epimers exerted some cannabimetic activity. Thus, apart from the 11-OH-Δ^8- and Δ^9-THC metabolites, the remaining metabolites may possess some Δ^9-THC-Iike action, but fairly high doses are needed to produce a cannabimetic effect. Thus, these data suggest that the contribution of most of these metabolites to the psychoactive properties of cannabis in natural settings is very minor. However, examinations of the chemical structure of these cannabinoids have helped to elucidate structural features contributing to cannabis intoxication.

L. ENDOCANNABINOID LIGANDS AND THE ECS

Given the existence of a specific recognition site to which Δ^9-THC binds, prompted the search for potential endogenous ligands for the CB_1R. One such molecule (AEA) was described in 1992 [29], soon followed by additional ligands such as 2-AG. Drug discrimination work thus far is limited to AEA and catalyzing enzymes, primarily FAAH, and putative transport mechanism(s). Initially, AEA was found to substitute for Δ^9-THC as well as for CP55,940, albeit only at doses that clearly affected response rate [256]. Attempts to replicate this finding in rats and monkeys failed [257–260]. Yet, inhibition of FAAH by URB597 [3-(3-carbamoylphenyl)phenyl] N-cyclohexylcarbamate] but not putative transport inhibition by AM404 [(5Z,8Z,11Z,14Z)- N-(4-hydroxyphenyl) icosa- 5,8,11,14-tetraenamide] and UCM707 [(5Z,8Z,11Z,14Z)-N-(3-furanylmethyl)-5,8,11,14-eicosatetraenamide]resulted in AEA substitution in Δ^9-THC trained rats; this AEA generalization was attenuated by rimonabant pretreatment [178]. Monkeys discriminating between i.v. administered vehicle and 0.1 mg/kg of Δ^9-THC showed rimonabant sensitive substitution in tests with AEA without concomitant FAAH inhibition [261]. Similarly, rats discriminating between i.p. administered AM356 and vehicle also showed generalization when evaluated 3 minutes (but not 15 minutes) post administration of AEA alone [104]. Accumulation of AEA due to FAAH inhibition by URB597

evaluated 40 and 120 minutes. after i.p. administered URB597 did not disclose generalization in Δ^9-THC (3.0 mg/kg) trained rats; AEA levels (but not 2-AG levels) in different rodent brain regions were significantly elevated at the 2 hr interval [262]; see also [263]. AEA is rapidly metabolized by FAAH and the very short duration of action makes pharmacological work with AEA difficult. Hence the need for ligands with more extended duration of action. Examples of such analogs include AM356, AM1346, 2-methylAEA [(5*Z*,8*Z*,11*Z*,14*Z*)-N-(2-fluoroethyl)-2-methylicosa-5,8,11,14-tetraenamide], O1812, AM881 [N-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; ACEA)] and ACPA [N-(cyclopropyl)-5Z,8Z,11Z,14Z-eicosatetraenamide]. With the exception of ACEA, which has not been tested in the cannabinergic drug discrimination model, all of these analogs have been found to substitute for Δ^9-THC in rats and monkeys [47, 48, 90, 104, 105, 107, 178, 257, 259–261], suggesting overlapping discriminative stimulus effects between Δ^9-THC and these AEA analogs. Three of the analogs (AM356, AM1346, and O1812) have also been established as the training stimulus and reciprocal cross-substitution between Δ^9-THC and the analogs was evident [104, 106, 107]. The binding affinities of these analogs for CB_1R is higher than that of Δ^9-THC, yet AM356, AM1346, and ACPA have consistently been found to be less potent in eliciting generalization with Δ^9-THC in rats and monkeys [47–49, 90, 104–106, 261, 264]. O1812 was found equipotent and 2-methylAEA three times more potent than Δ^9-THC in rats and monkeys, respectively [107, 257]. It should be noted that the analogs mostly have been evaluated using a single training dose of Δ^9-THC (or the analog), the exception being AM1346 [105, 106]. The studies employing AM356 as the training drug have used a single dose of 10 mg/kg. In an effort to remedy this limitation, new rats were subjected to discrimination training with 18 mg/kg, the acquisition of which is depicted in Figure 8-6. The study was terminated after six animals had died, and a seventh rat being on the verge of dying. The cause of toxicity was not determined. In our experience, the use of 10 mg/kg of AM356 as a training cue has never been associated with any apparent ill health effects.

Apart from relative potency differences between these analogs and Δ^9-THC, surmountable antagonism between the CB_1R antagonist rimonabant and the AEA analog AM356 has been difficult to demonstrate in rats [104, 106, 178], although the discriminative stimulus effects of AM356 clearly are blocked by CB_1R antagonists [49]; see also [110, 134]. In this regard, it is interesting that operant rate decreases produced by AM356 in CB_1R deficient mice were not antagonized by rimonabant [265]. Furthermore, cross-tolerance between Δ^9-THC and different AEA analogs varied with the sub-test of the tetrad assay employed [266]. However, vanilloid mechanism(s) do not appear directly involved in the discriminative stimulus effects of cannabinergics given that the TRPV1 antagonist capsazepine did not block the Δ^9-THC-like effects of AEA [178] and the TRPV1 agonist O1839 [(5Z,8Z,11Z,14Z)-*N*-(4-hydroxy-3-methoxybenzyl)-16,16-dimethyldocosa-5,8,11,14-tetraenamide] did not substitute for either Δ^9-THC or O1812 [107]. Monkeys maintained on i.v. infused 0.1 mg/kg Δ^9-THC readily disclosed surmountable antagonism with ACPA, AM356, as well as AEA (without FAAH inhibition), strongly supporting that CB_1R activation is the key component for shared discriminative stimulus effects between Δ^9-THC and the AEA analogs [261]; see also [90].

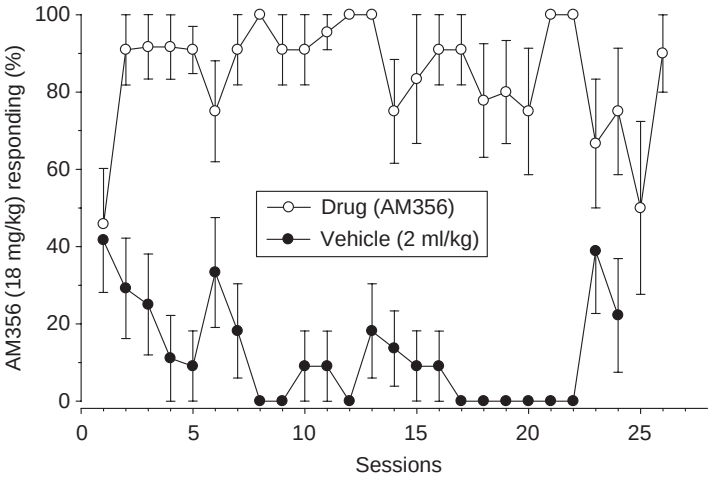


Figure 8-6. The graph illustrates percentage drug (AM356 = R-(+)-methanandamide) associated responding (based on the daily first choice) in rats subjected to discrimination training with AM356 (18 mg/kg) vs. vehicle, 20 min after i.p. administration. An operant (FR 10) two-lever choice procedure was used. Animals were trained for 20 min daily, 5 days a week. For simplicity, individual scores were rated as 100, 50, and 0 %. A score of 100% was defined as the first reinforcement being delivered after the animal had emitted 14 or less lever presses (FRF ≤ 14), that is, not pressing the "wrong/non-rewarding" bar more than 4 times before accumulating 10 responses on the "correct/rewarding," state appropriate lever. If exceeding 14 but less than 20 (FRF ≤ 19) lever presses before the 1st reinforcement delivery rendered a score of 50%, and if 20 or more presses occurred before the 1st reinforcement (45 mg food pellet, Bioserve), choice performance was scored as 0%. The initial group size was 12 male Sprague-Dawley rats (Taconic) approximately 3 months old upon arrival to the vivarium and after a week of acclimation, lever pressing was shaped by successive approximations and when stabilized at FR 10, drug discrimination training began. During the initial phase of discrimination training, only the state appropriate lever was available (the "wrong" lever retracted) and the dose of AM356 gradually increased from 5.6 to 10 (not shown) before the final dose of 18 mg/kg, at which point both levers were presented during the daily sessions of 20 min duration. The study was terminated after 27 "free-choice" sessions (both levers available for bar pressing) of alternating AM356 and vehicle pretreatment as 6 rats had died and a 7th rat was on the verge of dying. Hence, the number of observations per data point ranged from 12 animals at the 1st "free-choice" session to 5 rats at session 27. AM356 was synthesized at the Center for Drug Discovery (CDD), Northeastern University, Boston, MA. Unpublished data by Järbe, LeMay, Vadivel, and Makriyannis.

Compared to AM356, surmountable antagonism occurred more readily with AM1346-rimonabant combinations [105, 106]. Additionally, other work does not substantiate a significant role for CB_2R involvement in drug discriminations based on either CB_1R agonism (Δ^9-THC, ajulemic acid and AM356) or antagonism/inverse agonism (rimonabant) in monkeys and rats [49, 90, 110, 134, 218].

M. ECS INTERACTIONS WITH OTHER SIGNALING SYSTEMS

Given the role of endocannabinoids as regulators of neuronal signaling, interactions/cross-talk between ECS and other receptor systems seem likely. Two areas where drug discrimination methodology has been explored with cannabinergics concerns interactions between CB_1R activation/inactivation and opioids on the one hand and nicotine on the other hand; see, for example, [39, 41, 267, 268] for general overviews.

1. Opioid Ligands

In elegant studies, Solinas and colleagues suggested an opioid modulatory role in the expression of the discriminative stimulus effects of Δ^9-THC (3 mg/kg i.p.), particularly emphasizing μ receptor activity [167, 179]. As discussed earlier, opioid agonists do not cross-substitute with Δ^9-THC, nor do opioid antagonists block the effects of the training dose of Δ^9-THC [179, 269, 270]. Yet, the Δ^9-THC generalization gradient was shifted significantly to the left in the presence of morphine, heroin, and β-endorphin [but not in the presence of the preferential delta- and kappa agonists such as SNC-80 [4-[(R)-[(2S,5R)-4-allyl-2,5-dimethylpiperazin-1-yl](3-methoxyphenyl)methyl]-N,N-diethylbenzamide] and U50488 [2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1*R*,2*R*)-2-pyrrolidin-1-ylcyclohexyl]-acetamide], and to the right in the presence of preferential μ receptor antagonists such as naloxone and naltrexone, but not in the presence of opioid antagonists preferentially blocking delta-(naltrindole) and kappa (nor-binaltorphimine) opioid receptors. The augmentation of the discriminative stimulus effects of Δ^9-THC by heroin was blocked by naltrexone but the effects were not blocked by naltrindole and nor-binaltorphimine [167, 179]. Li et al. [269], working with monkeys trained to discriminate between vehicle and 0.1 mg/kg i.v. administered Δ^9-THC, failed to see augmentation (i.e., left-ward shift of the Δ^9-THC dose generalization curve) when combining Δ^9-THC and the opioid agonists morphine and heroin; all compounds were administered i.v. For monkeys trained with morphine (1.78 mg/kg; s.c.), Δ^9-THC pretreatment attenuated the morphine generalization curve. For monkeys maintained on twice daily morphine (5.6 mg/kg) and discriminating naltrexone (s.c.), Δ^9-THC (s.c.) treatment did not substitute for and did not attenuate the effects of naltrexone (morphine was shown to be effective). Δ^9-THC (s.c.) did not substitute for or attenuate the discriminative stimulus effects of midazolam in monkeys discriminating between 0.32 mg/kg s.c. administered midazolam and vehicle (with regard to midazolam, see also [271] using squirrel monkeys). Morphine induced antinociception (tail withdrawal) was, however, enhanced by Δ^9-THC [269]. The situation in humans is also complex and varied where naltrexone has been found to attenuate, enhance or exert no effect on various physiological and subjective effects of Δ^9-THC [123, 272–274].

2. Cholinergic Ligands

Using an approach similar to that described above, Solinas and colleagues [263, 275] suggested an important role also for nicotinic (n) α_7 receptors in the discriminative

and rewarding effects produced by CB_1R activation. Thus, the discriminative stimulus curves of Δ^9-THC and WIN55,212-2 were shifted to the right when these CB_1R agonists were co-administered with a cholinergic nicotinic (nACh) α_7 receptor selective antagonist (methyllicaconitine; MLA), but not when combined with the selective heteromeric non-α_7 nACh antagonist dihydrobetaerythrodine ($DH_\beta E$) [275]. Further involvement of ACh in the mediation of the discriminative stimulus effects of Δ^9-THC comes from studies showing that the nicotinic agonist nicotine as well as the muscarinic (m) agonist pilocarpine shifted the Δ^9-THC generalization curve to the left. These leftward shifts were blocked by mecamylamine (a nACh antagonist) and scopolamine (a mACh antagonist), employing doses of the cholinergic antagonists not blocking the discriminative stimulus effects of the training dose of 3 mg/kg of Δ^9-THC. The augmentation by nicotine (but not by pilocarpine) was blocked by rimonabant, suggesting indirect mediation, possibly by endogenous formation by AEA. Support for such possibility was derived from studies employing the FAAH inhibitor URB597. FAAH inhibition augmented the Δ^9-THC-like effects of nicotine in a rimonabant sensitive manner, that is, the URB597-induced facilitation of nicotine generalization was attenuated by rimonabant [263]. Unfortunately, this emphasis on the significance of particularly nACh related augmentation seems at odds with another study using rats trained to discriminate between s.c. administered 0.4 mg/kg of nicotine and vehicle. Neither CB_1R activation by WIN55,212-2, CP55,940 and AEA, nor AEA accumulation by URB597 or AM404 (both with and without 2.5 mg/kg of AEA) resulted in generalization to the nicotine cue, nor were there significant changes in the nicotine curve when the cannabinoid ligands were co-administrated with different doses of nicotine. CB_1R and CB_2R antagonism failed to generalize to the nicotine cue. Unlike above data, $DH_\beta E$ antagonized the nicotine cue whereas MLA did not [276].

It has been suggested that perhaps rewarding and discriminative stimulus effects of Δ^9-THC are differentially modulated by cholinergic mechanisms [277], but no doubt careful attention is required to understand these seemingly disconcordant outcomes. Although we have come accustomed to the notion that drug discrimination is a very selective and pharmacologically specific behavioral end-point, the ECS, by virtue of its endogenous ligands being synthesized and released "on demand," may be much more dependent on contextual circumstances than is the case for more classical transmitter systems.

N. CONCLUSIONS/SUMMARY

To summarize, Δ^9-THC, the main active constituent in marijuana preparations, has had quite a long tradition serving a cueing function in different drug discrimination tasks and laboratory animals (rats, monkeys, mice, gerbils, and pigeons) as well as man. The first two drug discrimination reports appeared in 1972. A survey of the drug discrimination literature revealed that Δ^9-THC has been the most commonly used cannabinergic training drug irrespective of species and that rats have been the most studied species. Other cannabinergics used for training have been CB_1R agonists such as CP47,497,

CP55,940, WIN55,212-2, BAY38-7271, and BAY59-3074 as well as AEA analogs such as AM356, AM1346, and O1812. The general observation that the drug discrimination procedure is pharmacologically specific seems to hold true also for cannabinergics across different training doses and CB_1R ligands although some studies have noted increased levels of "Δ^9-THC-like" responding in tests with CNS "depressant" drugs, in particular the benzodiazepine diazepam. However, as of yet only agonists activating central CB_1R have been found to show complete substitution for Δ^9-THC and other cannabinergics with relative potencies reflective of their affinity for CB_1R in binding assays, exceptions being analogs derived from the AEA template such as *R*-(+)-methanandamide, arachidonylcyclopropylamide, and AM1346. These latter analogs all have higher binding affinity for the CB_1R as well as enhanced receptor subtype selectivity (CB_1/CB_2), yet exhibit less relative potency compared to Δ^9-THC. Although CB_2R activation / inactivation regarding CB_1R mediation of drug discrimination effects of CB_1R ligands are unknown, tests with CB_2R agonists and antagonists consistently have failed to substitute or antagonize, respectively, drug discriminations maintained by CB_1R agonists as well as the CB_1R antagonist/inverse agonist rimonabant in rats and monkeys. The discriminative stimulus functions of rimonabant were evaluated using a taste aversion approach. Attempts to establish rimonabant as a discriminative stimulus using operant methodology for pigeons, rats, and monkeys have been unsuccessful thus far. However, monkeys pretreated with Δ^9-THC prior to rimonabant administration readily acquired the discrimination. The potential contribution of metabolites in cannabinergic drug discrimination has only been examined regarding Δ^9-THC. For example, (-)-11-OH- Δ^8-THC is a potent metabolite that readily substitutes for Δ^9-THC in a stereo selective manner, that is, the stereo isomer (+)-11-OH-Δ^8-THC does not elicit cue effects similar to Δ^9-THC. Of other metabolites examined, only 8β,11 di-OH-Δ^9-THC produced complete generalization to the Δ^9-THC stimulus by pigeons but with a relative potency considerably less than that of Δ^9-THC. Stereoselectivity for eliciting cannabinergic-like activity has been studied with other CB_1R agonists as well, notably the enantiomeric pair HU210 [(-)-11-OH-Δ^8-THC-DMH] and HU211 [(+)-11-OH-Δ^8-THC-DMH]. Among the phytocannabinoids [i.e., C_{21} terpenophenolic cannabinoids present in plant material(s)] examined with drug discrimination, CBN but not CBD, substituted for and also acted additively with the Δ^9-THC discriminative stimulus but exhibiting lesser relative potency. CBD neither substituted for, nor antagonized the discriminative stimulus effects of Δ^9-THC. At higher doses, CBD may prolong the discriminative stimulus effects of Δ^9-THC, possibly by liver metabolic, enzymatic competition. Studies on interactions between the discriminative stimulus effects of Δ^9-THC and opioid as well as cholinergic ligands have produced variable results. Some studies suggested enhancement / diminution of the Δ^9-THC discriminative stimulus by μ opioid receptor activation / inactivation (rats), whereas another study (monkeys) found no such evidence. Results from rats discriminating between Δ^9-THC and vehicle suggested a modulatory role by nicotinic cholinergic mechanism(s), possibly indirectly through enhanced AEA formation in brain. However, rats discriminating between nicotine and saline generated no evidence to support any involvement by ECS in the nicotine discriminative stimulus.

O. ADDENDUM

As of April 2010, four research reports concerning the discriminative stimulus effects of cannabinoid related ligands have been published since the literature search dating back to July / August 2009. One recent study showed that a selective and efficacious dual FAAH/MAGL inhibitor resulted in generalization to Δ^9-THC (10 mg/kg) in thus trained mice and also showed broad activity in the tetrad test for CB_1 agonism. Selectively augmenting the levels of either endocannabinoid separately by FAAH inhibition (AEA) or MGL inhibition (2-AG) did not result in a cannabimimetic-like state [278]. Another study reported enhancement of the discriminative stimulus effects of Δ^9-THC by indirect (cocaine and amphetamine) and direct (quinpirole and apomorphine) acting dopamine 2 receptor agonists, postulating that release of endogenously formed AEA by activation of dopamine 2 receptors was the mediator for the outcome. When examined alone, none of these dopamine 2 receptor agonists substituted for 3 mg/kg Δ^9-THC [279]. A third study examined substitution by cannabinergic aminoalkylindoles [WIN55,212-2 and AM678 [naphthalene-1-yl(1-pentyl-1H-indol-3-yl)methanone] and their blockade by rimonabant in two groups of rats discriminating between vehicle and 1) AM356 (10 mg/kg), or 2) Δ^9-THC (1.8 mg/kg). Differences in the substitution/blocking patterns were suggested to be due to postulated differences in the ligand-receptor binding motifs by AM356 and Δ^9-THC. Alcohol did not substitute in either of the two groups [280]. The fourth study evaluated the reinforcing and discriminative stimulus effects of taranabant [*N*-[(1S,2S)-3-(4-chlorophenyl)-2-(3-cyanophenyl)-1-methylpropyl]-2-methyl-2-{[5-(trifluoromethyl)pyridin-2-yl]oxy}propanamide; MK-0364], a potent and selective CB_1R antagonist/inverse agonist. The drug did not sustain self-administration in monkeys and did not substitute for Δ^9-THC in rats trained to discriminate between Δ^9-THC (3 mg/kg) and vehicle [281].

Acknowledgments I thank R. Gifford, B. LeMay, and S. Tai for comments on earlier drafts of this manuscript. Thanks are extended to S.K. Subramanian and particularly K. Vemuri for their help with chemistry. The work was partly funded by grants from NIH/NIDA awarded to the Center for Drug Discovery, Northeastern University, Boston, MA (DA 09064, 00253, 9158, and 7215) as well as a grant from Peter F. McManus Charitable Trust.

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9

DISCRIMINATIVE STIMULUS PROPERTIES OF RECEPTOR ANTAGONISTS

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

A. INTRODUCTION

As can be seen in the other chapters in this book and in a casual perusal of the training drugs listed on the Drug Discrimination Database (http://www.drugrefs.org/), the majority of drug discrimination studies have used receptor agonists or partial agonists as the training drug; receptor antagonists are typically tested in combination with the training drug. The ability of selective antagonists to block the training drug's cue is often used to establish the underlying mechanism of action (i.e., the neurotransmitter receptor) that mediates the discriminative stimulus properties of that drug. While more limited, there have been a number of studies that have examined the discriminative stimulus properties of receptor antagonists. The present chapter will provide a selective review of these studies. To facilitate this process, the chapter is organized both in terms of specific receptor mechanisms and drug classes, as appropriate. Typically only a few antagonists have been tested within a specific category with a fairly limited number of studies. Another limitation of studying receptor antagonists in the drug discrimination paradigm is that it is very difficult to antagonize (block or attenuate) the discriminative stimulus properties of a receptor antagonist without using higher doses of a receptor agonist that also disrupt response rates of the animals. Unfortunately, once unconditioned drug effects are apparent (i.e., response rate disruption), it becomes very difficult to interpret any attenuation of the training drug's discrimination stimulus.

This chapter will not review the extensive literature on glutamatergic receptor antagonists (primarily the uncompetitive NMDA antagonists phencyclidine [PCP], ketamine, and MK-801). A search on the Drug Discrimination Database revealed over 300 articles (over 200 on PCP alone) in which one of these drugs was either the training drug or a primary drug of interest in a drug discrimination study. Several excellent reviews have been written on the discriminative stimulus properties of NMDA antagonists [e.g., 1–3] and interest in the discriminative stimulus properties of NMDA antagonists shows no sign of abating [e.g., 4, 5]. There is considerable interest in the therapeutic use of NMDA antagonists, but these compounds may produce undesirable side-effects similar to those associated with the uncompetitive antagonist PCP. A good example of research in this area is a recent study by Nicholson and Balster [4]. In order to evaluate the PCP-like properties of a series of glycine-site partial agonists and antagonists,

Nicholson and Balster conducted a drug discrimination study in which rats were trained to discriminate PCP (2.0 mg/kg, i.p.) from saline or the competitive NMDA antagonist NPC 17742 [2*R*,4*R*,5*S*-(2-amino-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid)] (4.0 mg/kg, i.p.) from saline. None of the glycine-site partial agonists (aminocyclopropanecarboxylic acid methyl ester and (+)-HA-966) nor the glycine-site antagonists (L701,324; MDL 100,458; MDL 100,748; MDL 103,371; MDL 104,472; MDL 105,519; MRZ 2/571; MRZ 2/576; and ACEA 0762) substituted for PCP. All of these compounds produced less than 50% PCP-appropriate responding. In the NPC 17742-trained rats, similar results were obtained with the exception of one compound, ACEA 0762 (5-nitro-6,7-dimethyl-1,4-dihydro-2,3-quinoxalinedione), which fully substituted for NPC 17742. On the basis of these results, Nicholson and Balster concluded that NMDA glycine-site partial agonists and antagonists do not share the subjective effects of NMDA channel blockers (PCP) or competitive antagonists (NPC 17742). The authors also suggested that these compounds were less likely to produce undesirable behavioral effects clinically. This study is a good example of the utility of the drug discrimination procedure for the preclinical assessment of potential therapeutic drugs.

Finally, the present chapter presents selected, representative studies for several drug classes and for a number of neurotransmitter receptor systems. As one might expect there is sometimes overlap and this classification approach does not produce mutually exclusive categories. Also, the emphasis is on drug discrimination studies that primarily used two-lever operant procedures, but there are a few three-choice studies discussed. Findings from other drug discrimination procedures, T-maze (see review by Overton [6]) or taste-aversion drug discrimination procedures (see review by Riley [7]), will be mentioned as appropriate. Two of the earliest reports of operant drug discrimination procedures involved the training of rats to discriminate ethyl alcohol from saline [8, 9] and the muscarinic antagonist atropine sulfate from saline [8]. A chapter by Harris and Balster [10] titled "An analysis of the function of drugs in the stimulus control of operant behavior" reported the first systematic study of the two-lever operant procedure for drug discrimination that has since become the standard procedure for most drug discrimination studies. They used various schedules of reinforcement, including CRF (continuous reinforcement, which they found was not a good reinforcement schedule to use), DRL (differential reinforcement of low rates of responding), and FR (fixed ratio), which is used in the majority of operant drug discrimination studies today (for additional discussion of schedules of reinforcement, see Chapter 2). While the dopamine (DA) agonist d-amphetamine was the drug used in the majority of their studies, they did test the muscarinic cholinergic antagonist atropine, which displayed good discriminative stimulus control of operant responding. Interestingly, they were not able to establish methyl atropine (which does not readily cross the blood-brain barrier) as a discriminative stimulus indicating that atropine's discriminative stimulus was centrally mediated.

B. ADRENOCEPTOR ANTAGONISTS

The use of adrenoceptor antagonists as training drugs has been mostly limited to the use of the α_2-adrenoceptor antagonist yohimbine, although one study [11] has used

idazoxan (also an α_2-adrenoceptor antagonist) and one recent study [12] successfully trained the β-adrenoceptor antagonist *S*(-)propranolol. Interestingly, there have been no reported attempts to train the α_1-adrenoceptor antagonist prazosin in the drug discrimination procedure, although prazosin has been used extensively to explore the role of α_1-adrenoceptors in the discriminative stimulus properties of many drugs.

1. Yohimbine

The first study to establish yohimbine as a discriminative stimulus was by Winter [13]. He trained rats to discriminate 3.0 mg/kg (i.p.) of yohimbine from saline in a mean of 33 sessions. Substitution testing with d-amphetamine (DA agonist), harmaline (MAO inhibitor), and LSD (5-HT receptor agonist) failed to produce yohimbine-appropriate responding with intermediate levels of responding. The yohimbine discriminative cue was not blocked by antagonism at serotonergic (pizotifen [BC-105]), α-adrenergic (phentolamine), or dopaminergic (butaclamol) receptors.

Several studies have examined the role of serotonergic mechanisms in the discriminative stimulus properties of yohimbine, a drug that is typically regarded as a selective α_2-adrenoceptor antagonist. Winter and Rabin [14, see also 13] found cross-generalization between yohimbine (6.0 mg/kg training dose, i.p.) and the 5-HT_{1A} agonists 8-OH DPAT (8-hydroxy-2-(di-*n*-propylamino)tetralin) (0.2 mg/kg training dose, i.p.) and ipsapirone (10 mg/kg training dose, i.p.). They also reported that dissociation constants for 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 binding sites (K_D values in nM) revealed a high affinity of 8-OH DPAT and ipsapirone at 5-HT_{1A} sites (19.6 and 36.1 nM, respectively), but little affinity with yohimbine (1,336 nM). Additional evidence for serotonergic activity in yohimbine's discriminative stimulus was reported by Colpaert [15], who reported cross-generalization between rats trained to discriminate either the serotonergic agonist LSD (0.16 mg/kg) or yohimbine (5 mg/kg) from saline. Cross-generalization between two training drugs is typically taken as evidence that the two drugs share discriminative stimulus properties and that the underlying receptor mechanisms may be similar [16].

Further evidence for the role of serotonergic 5-HT_{1A} antagonism in the discriminative stimulus properties of yohimbine was found in a subsequent study by Winter and Rabin [17]. They trained one group of rats to discriminate yohimbine (3 mg/kg, i.p.) from saline and another group was trained to discriminate 8-OH DPAT (0.2 mg/kg, i.p.) from saline. They found that 8-OH DPAT fully generalized to the α_2-adrenoceptor antagonists yohimbine, rauwolscine, and L-657,743, but not to idazoxan or atipamezole. Dissociation constants (K_D, nM) at the 5-HT_{1A} receptor for these drugs were 52, 84, 91, 199, and 13,000, respectively. Based on these results the authors concluded that the generalization of 8-OH DPAT to α_2-adrenoceptor antagonists could be attributed to antagonism of 5-HT_{1A} receptors. Further support for this conclusion came from the generalization of yohimbine's discriminative cue to the 5-HT_{1A} agonists 8-OH DPAT, flesinoxan, and tandospirone. Flesinoxan and tandospirone have negligible affinity for α_2-adrenceptors, but have a high affinity for 5-HT_{1A} receptors. Based on these results, Winter and Rabin cautioned that the use of yohimbine for the assessment of α_2-adrenoceptor function must consider that yohimbine may also be producing effects via 5-HT_{1A} receptors [see also 18]. It would be interesting to test

5-HT_{1A} receptor antagonists such as WAY 100635 ([*Carbonyl*-^{11}C]*N*-(2-(1-(4-(2-methoxyphenyl)-piperazinyl)ethyl)-*N*-pyridinyl) cyclohexanecarboxamide) or NAN-190 (1-(2-methoxyphenyl)-4-(4-phthalimidobutyl)piperazine) to help resolve the role of 5-HT_{1A} in yohimbine's discriminative stimulus cue. These studies demonstrate how the drug discrimination procedure may be used to determine the underlying receptor mechanisms that mediate the discriminative stimulus properties of a drug.

2. Idazoxan

The other α_2-adrenceptor antagonist that has been trained as a discriminative stimulus is idazoxan. Sanger [11] trained rats to discriminate 10 mg/kg (i.p.) of idazoxan from saline and found dose-dependent generalization to idazoxan and yohimbine. In substitution tests, the α_1-adrenoceptor agonist cirazoline, the α_1-adrenoceptor antagonist prazosin, and the α_2-adrenoceptor agonist clonidine did not substitute for idazoxan. In addition, it was found that prazosin and clonidine did not block the idazoxan discriminative cue. The anxiolytics buspirone and ipsapirone generated high levels of idazoxan-appropriate responding, but only at doses that decreased response rates. While Sanger concluded that the idazoxan discriminative stimulus is probably mediated by antagonism at α_2-adrenoceptors, he was not able to explain the inability of clonidine to block the idazoxan discriminative cue.

3. Propranolol

While the β-adrenoceptor antagonist propranolol has often been used in drug discrimination studies to help evaluate the relative contribution of α- and β-adrenoceptors in the discriminative stimulus properties of many compounds, Young and Glennon's [12] recent study is the first to evaluate the discriminative stimulus properties of propranolol directly as the training drug. They chose the *S*(-)propranolol isomer as the training drug as it has been shown to be approximately 100 times more potent than the *R*(+)propranolol isomer at β-adrenoceptors [19] and is presumably the pharmacologically relevant enantiomer. They were able to successfully train rats to discriminate a dose of 5 mg/kg *S*(-)propranolol (i.p.) from saline in approximately 55 training sessions. A time-course study showed that the 15-minute pre-session injection time produced maximal responding on the drug lever (~100% drug lever responding [%DLR]) and that at 60 minutes the rats still displayed 91% DLR. However, as the injection time was extended the %DLR decreased below 80% DLR at 90 minutes and longer intervals. Thus, *S*(-)propranolol appeared to be rapidly absorbed and its duration of effect was relatively short. The *S*(-)propranolol discriminative cue generalized to both the racemic mixture of propranolol and to the *R*(+)propranolol isomer, but it was approximately two times more potent than the racemic mixture and four times more potent than the *R*(+) isomer. A series of substitution tests with adrenoceptor and serotonergic agents and the nonselective monoamine reuptake inhibitor cocaine were conducted. They reported that the α_1-adrenoceptor prazosin blocked the discriminative stimulus of *S*(-)propranolol completely and competitively. In characterizing the discriminative cue of *S*(-)propranolol, Young and Glennon concluded that it was centrally mediated, dose-related, time

dependent, and stereoselective. As *S*(-)propranolol generalized to the nonselective 5-HT agents TFMPP (3-Trifluoromethylphenylpiperazine) and RU 24069 (5-methoxy-3-(1,2,3,6-tetrahydro-4-pryridyl)-1*H*-indole), serotonergic mechanisms may play a role in *S*(-)propranolol's discriminative cue. In addition, the ability of the α_1-adrenoceptor prazosin to block *S*(-)propranolol's discriminative cue implicates an increase in α_1-adrenoceptor activity by *S*(-)propranolol. In a separate group of rats trained to discriminate cocaine (8 mg/kg, i.p.), the cocaine discriminative cue fully generalized to *S*(-)propranolol, but was only partially blocked by prazosin. Thus, while *S*(-)propranolol and cocaine cross-generalized, their discriminative cues were differentially antagonized by prazosin. Based on these results, Young and Glennon suggested that propranolol might be best characterized as a partial agonist at adrenoceptors.

C. ANTIHISTAMINES

The first drug discrimination study with antihistamines was conducted by Overton [20], who investigated the discriminative stimulus properties of several antihistamines in a shock-escape T-maze task and concluded that H_1 histamine receptor antagonists seemed to be fairly specific in their discriminative stimulus properties. This conclusion was based on the finding that the H_1 antagonists tested in that study (diphenhydramine, pyrilamine [also known as mepyramine], and diminhydrinate [a mixture of diphenhydramine and the stimulant 8-chlorotheophylline]) generalized to each other, but not to drugs from other drug classes. Another characteristic of antihistamines is that they possess sedative properties. Winter [21] used a two-lever drug discrimination procedure to see whether the sedative properties of antihistamines are distinct from their discriminative stimulus properties. He trained rats to discriminate either 10 mg/kg (i.p.) of diphenhydramine or 10 mg/kg (i.p.) of chlorpheniramine from saline. Diphenhydramine was chosen as it has strong sedative properties and chlorpheniramine was chosen because it has relatively less sedative properties [see 22]. While a relatively small number of rats were used in each group (N = 6), there were marked differences in the acquisition of stimulus control for each drug. The diphenhydramine-trained rats (all 6) met the training criterion in a mean of 26 sessions; whereas only 4 of 6 rats in the chlorpheniramine group met the training criterion in a mean of 56 sessions. Winter abandoned training with chlorpheniramine after 75 sessions for the remaining two rats. While clearly requiring a greater number of training sessions, the chlorpheniramine cue appeared to be stable once acquired. Both drugs displayed dose-dependent generalization functions, with chlorpheniramine appearing to be slightly more potent as its ED_{50} was slightly less than 2.0 mg/kg and diphenhydramine's ED_{50} was slightly greater than 3 mg/kg (my estimates from the graphs). Cross-generalization testing found that chlorpheniramine fully substituted for diphenhydramine, but diphenhydramine only partially substituted for chlorpheniramine producing a maximum of approximately 65% chlorpheniramine -appropriate responding. Winter then evaluated two other antihistamines that varied in their sedative properties—a more sedative drug, promethazine, and a less sedative drug, azatidine. Both of these drugs fully substituted for diphenhydramine and chlorpheniramine. While Winter concluded that the sedative properties of antihista-

mines in humans are not correlated with their discriminative stimulus properties in rats, it could be argued that the relative differences in sedative properties might have played a role in the acquisition of the discriminative cue for each drug and the asymmetrical cross-generalization between these two antihistamines.

In another study of the discriminative stimulus properties of antihistamines, White [23] trained rats to discriminate 10 mg/kg (i.p.) of mepyramine (pyrilamine) from saline in rats and found that the mepyramine cue generalized to the antihistamines tripelennamine and chlorpheniramine (>90% mepyramine-appropriate responding). While the anticholinergic drug scopolamine and the local anesthetic procaine did not substitute for mepyramine, the antidepressant imipramine (which displays a strong binding affinity for H_1 receptors [24, 25]) fully substituted for mepyramine. These findings suggested that the discriminative stimulus properties of mepyramine are mediated by antagonism of H_1 histamine receptors.

Antihistamines also have been trained in pigeons as discriminative stimuli. Karas et al. [26] trained pigeons to discriminate 5 mg/kg of tripelennamine from saline. They reported full substitution for the antihistamines diphenhydramine and pyrilamine and partial substitution for chlorpheniramine and promethazine. The nonantihistaminergic drugs chlorpromazine, cimetidine, d-amphetamine, diazepam, morphine, pentazocine, phenobarbital, and sodium valproate failed to produce any significant levels of tripelennamine-appropriate responding. The H_1 histamine antagonists chlorpheniramine (3 mg/kg in four pigeons and 5.6 mg/kg in one pigeon, i.m.) and promethazine (3 mg/kg, i.m.) also have been established as discriminative stimuli in pigeons [27]; however, these drugs fail to cross-generalize to each other. Different substitution patterns were also found for several H_1 antagonists that were tested. Tripelennamine fully substituted for chlorpheniramine, but not for promethazine, whereas diphenhydramine substituted for promethazine, but not for chlorpheniramine. The dopamine agonist d-amphetamine fully substituted for chlorpheniramine in 2 of 4 pigeons and partially in one other pigeon and in 1 of 4 pigeons in the promethazine-trained pigeons. These findings, in contrast to those for mepyramine (pyrilamine) [23], suggest that the discriminative stimulus properties of antihistamines may not depend entirely on antagonist activity at H_1 histamine receptors. Additional support for this idea comes from studies in which several antihistamines have been found to substitute for the dopamine agonists d-amphetamine [28] and cocaine [29] and cocaine and amphetamine fully substituted for the over-the-counter drug mixtures, dextromethorphan + ephedrine and dextromethorphan + diphenhydramine [30].

It is also possible that differences between species (pigeons versus rats) might explain some of the discrepant findings discussed above and further evidence for species differences in the discriminative stimulus properties of antihistamines was found in a study by Evans and Johanson [28]. In pigeons trained to discriminate 1.0 mg/kg (i.m.) of d-amphetamine, the antihistamines tripelennamine, diphenhydramine, and chlorpheniramine fully substituted for the d-amphetamine cue; whereas, promethazine did not. In rhesus monkeys trained to discriminate either 1.0 mg/kg or 0.56 mg/kg (oral gavage) d-amphetamine, only tripelennamine substituted for d-amphetamine. Regardless of species differences, it is clear that the discriminative stimulus properties of antihistamines are not based totally on their antagonist activity at H_1 histamine receptors.

D. ATYPICAL ANTIPSYCHOTIC DRUGS

There has been more research on the discriminative stimulus properties of antipsychotic drugs than any of the other drug classes and receptor mechanisms covered in this chapter. Goudie and Smith [31] and more recently Porter and Prus [32] have provided comprehensive reviews of the discriminative stimulus properties of antipsychotic drugs; therefore, the present review will provide only a summary of their major findings and conclusions. Also, I have chosen to describe the discriminative stimulus properties of the typical (first generation) antipsychotic drugs haloperidol and chlorpromazine in the section on dopamine antagonists as they are often characterized in this manner. Finally, in order to limit this review somewhat, this section will focus on the discriminative stimulus properties of the atypical (second generation) antipsychotic drug clozapine. Information about the discriminative stimulus properties of olanzapine, quetiapine, and ziprasidone can be found in the reviews by Goudie and Smith [31] and Porter and Prus [32].

As described in the reviews by Goudie and Smith [31] and Porter and Prus [32], clozapine is the gold standard against which newer atypical antipsychotic drugs have been compared. Clozapine displays robust discriminative stimulus properties in both two-choice and three-choice drug discrimination procedures and has been established in rats (the majority of the studies), mice (three studies) [33–35], pigeons (one study) [36], and squirrel monkeys (one study) [37].

The dibenzodiazepine clozapine differs from typical antipsychotic drugs such as haloperidol (a butyrophenone) and chlorpromazine (a phenothiazine) in that it displays a relatively low binding affinity to dopamine D_2 receptors. Clozapine also possesses a very diverse binding profile and displays a high binding affinity for dopaminergic D_1, D_4, serotonergic 5-$HT_{2A/2C}$, 5-HT_6, 5-HT_7, cholinergic m_1, m_2, m_3, m_4, adrenergic α_1, α_2, and histaminergic H_1 receptors [38–41]. While clozapine is usually characterized as an antagonist at these receptors, it does display weak partial agonist activity at m_1 receptors [42, 43] and agonist activity at muscarinic m_4 and serotonergic 5-HT_{1A} receptors [38, 44, 45]. This very diverse binding profile has made it difficult to determine the underlying mechanisms of clozapine's discriminative stimulus. Also, there are differences in the discriminative stimulus effects of clozapine between rats, mice, and pigeons.

In rats the only receptor mechanism that has consistently elicited clozapine's discriminative stimulus is antagonism of cholinergic muscarinic receptors. Nielsen [46] was the first to report that antagonism of muscarinic receptors was sufficient to elicit clozapine appropriate responding in rats trained to discriminate 5.76 mg/kg (i.p.) of clozapine from vehicle. Specifically, the muscarinic antagonists scopolamine and atropine fully substituted for clozapine. Nielsen also found that the muscarinic agonist oxotremorine attenuated clozapine-appropriate responding and this finding has been replicated by Kelley et al. [47, see Table 9-1]. Goudie et al [48] replicated these findings for scopolamine in a study that tested a large number of selective receptor ligands. Again, the only selective ligand that fully substituted for clozapine was scopolamine [see also 49].

TABLE 9-1. Receptor antagonists used as training drugs in drug discrimination studies

DRUG CLASS	REFERENCES
ADRENOCEPTOR ANTAGONISTS	
S(-)propranolol (B-adrenoceptor antagonist)	Young and Glennon 2009 [12]
Yohimbine (α_2-adrenoceptor antagonist)	Browne 1981 [148];Colpaert 1984 [15]; Palumbo and Winter 1992 [18]; Winter 1978 [13], 1989 [149], 1992, 1993; Winter and Rabin 1989 [14], 1992 [17]
Idazoxan (α_2-adrenoceptor antagonist)	Sanger 1989 [11]
ANTIHISTAMINE DRUGS	
Diphenhydramine	Winter 1985 [21]
Chlorpheniramine	Winter 1985 [21]; Evans et al. 1991 [27]
Mepyramine (pyrilamine)	White 1985 [23]
Tripelennamine	Karas et al 1985 [26]
Promethazine	Evans et al. 1991 [27]
ATYPICAL ANTIPSYCHOTIC DRUGS	
Clozapine	see reviews by Goudie & Smith 1999 [31] and Porter & Prus 2009 [32]
Olanzapine	Porter & Strong 1996 [150]; Porter et al. 2000 [151]
Quetiapine	Goudie et al. 2004 [147]; Smith & Goudie 2002 [152]
Ziprasidone	Wood et al. 2007 [153]
BENZODIAZEPINE ANTAGONISTS	
Flumazenil (Ro 15-1788)	De Vry and Slangen 1985 [61]; France and Gerak 1997 [154] ; Gerak and France 1999 [155]; Koek et al. 2006 [64]; McMahon et al. 2002 [ ]; Rowan and Lucki 1992 (Conditioned Taste Aversion Discrimination) [157]; Smith and Bickel 1999 [65]; Woudenberg and Slangen 1990 [62]
CANNABINOID ANTAGONISTS	
SR 141716A (Rimonabant) (CB1 antagonist)	Järbe et al. 2004 [70], 2008 [71]; Mansbach et al. 1996 [68]; McMahon and France 2003 [73]; McMahon 2006 [74]; Pèrio et al. 1996 [69]
CHOLINERGIC ANTAGONISTS	
Atropine (muscarinic antagonist)	Harris & Balster 1971 [10]; Kubena & Barry 1969b; Overton 1977 (T-Maze) [77]
Scopolamine (muscarinic antagonist)	Jung et al. 1988 [75]; Kelley et al. 1995 [81]; Kelley and Porter 1997 [50]; Overton 1977 (T-Maze); Witkin et al. 1992 [158]
DOPAMINE ANTAGONISTS	
Chlorpromazine (Typical Antipsychotic Drug)	Barry et al. 1974 [92]; Goas & Boston 1978 [93]; Harris & Balster 1971 [10]; Overton 1966 (T-Maze) [91]; Porter et al. 1998 [94]; Porter et al. 2005 (3-choice study) [95]; Stewart 1962 [90]

(*Continued*)

TABLE 9-1. (*Continued*)

DRUG CLASS	REFERENCES
Haloperidol (Typical Antipsychotic Drug)	Barrett et al. 2001 [101], 2004 [100]; Barrett et al. 2005 (3-choice study) [107]; Caul et al. 1996 [102], 1997 (3-choice study) [103]; Colpaert et al. 1976 [96]; Gauvin et al. 1994 [104], 1997 (3-choice) [105]; Haenlein et al. 1985 (3-choice study) [99]; McElroy et al. 1989 [97]; Stadler et al. 1999 [106]; Wiley & Porter, 1993 [56]
Tiapride (selective D_2/D_3 antagonist)	Cohen et al. 1997 [108]
PNU-99194A (selective D_3 antagonist)	Baker et al. 1997 [159]; Franklin et al. 1998 [160]
GABA ANTAGONISTS	
Pentylenetetrazol ($GABA_A$ antagonist)	Jung et al. [78] review by Jung et al. 2002 [109]
OPIATE ANTAGONISTS	
Naloxone (mu, kappa, delta antagonist)	Carter and Leander 1982 [115]; Lal et al. 1978 [111]; Miksic et al. 1981 [113]; Overton and Batta 1979 (T-Maze) [114]; Weissman 1978 [112] Conditioned Taste Aversion Discrimination Davis et al. 2009 [122]; Kautz et al. 1989 [120]; Riley 1997 (Review) [7]; Smurthwaite et al. 1992 [121]
Naltrexone (mu, kappa, delta antagonist)	France and Woods 1987 [125] (3-choice), 1988 [127], 1989 [129]; Gellert and Holtzman 1979 [124]; Sell and France 2002 [128]; Valentino et al. 1983 [126]
Diprenorphine (mu, kappa, delta antagonist)	DeRossett and Holtzman 1986 [132]; Smurthwaite and Riley 1992 (CTA Discrimination) [133]
Nalorphine (mixed agonist/antagonist)	Hirschhorn 1977 [134]; Smurthwaite and Riley 1993 (CTA Discrimination) [133]
SEROTONERGIC ANTAGONISTS	
Mianserin (tetracyclic antidepressant)	Kelley et al. 1995 [81]
Pizotifen	Minnema et al. 1984 [139]
MDL100907 (selective 5-HT_{2A} antagonist)	Dekeyne and Millan 2002 [142], 2003 [143]
WAY 100635 (5-HT_{1A} antagonist/D_4 agonist)	Marona-Lewicka and Nichols 2009 [146]

The role of muscarinic antagonism in clozapine's discriminative cue was more fully explored by Kelley and Porter [50]. They trained one group of rats to discriminate 5 mg/kg (i.p.) of clozapine from vehicle and another group to discriminate 0.125 mg/kg (i.p.) scopolamine from vehicle and found that clozapine and scopolamine displayed cross-generalization to each other—a finding that suggests that the discriminative stimulus properties of the two drugs may be mediated via a common mechanism [see

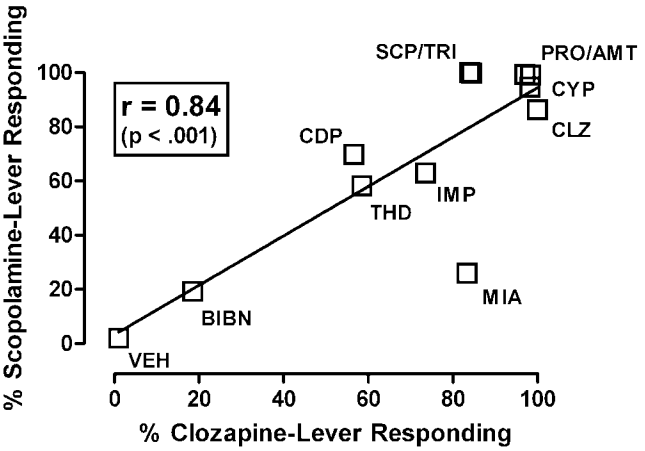

Figure 9-1. Data adapted from Kelley and Porter [50] are summarized for a series of drugs tested in one group of rats trained to discriminate 5.0 mg/kg clozapine from vehicle and in another group of rats trained to discriminate 0.125 scopolamine from vehicle in a two-lever operant task. The highest percent of scopolamine-lever responding is shown on the Y axis as a function of the highest percent of clozapine-lever responding for each drug on the X axis. The regression line for the data and the correlation coefficient are also shown. Abbreviations: Amitriptyline (AMT); BIBN-99 (BIBN); Chlordiazepoxide (CDP); Clozapine (CLOZAPINE); Cyproheptadine (CYP); Imipramine (IMP); Mianserin (MIA); Promethazine (PMZ); Scopolamine (SCP); Thioridazine (THD); Trihexyphenidyl (TRI); Vehicle (VEH).

16]. Kelley and Porter also found that the m_1 muscarinic antagonist trihexyphenidyl fully substituted for both clozapine and scopolamine; whereas, the m_2 antagonist BIBN 99 did not substitute for either drug. Using data from the Kelley and Porter [50] study (see Figure 9-1), the highest percent drug-lever responding for clozapine (X axis) is plotted as a function of the highest percent drug-lever responding for scopolamine (Y axis) for the drugs tested in that study. As can be seen, there was a significant positive correlation ($r = 0.84$, $p < 0.001$) between percent clozapine-responding and percent scopolamine responding. Also, the drugs with higher binding affinities at muscarinic receptors generally produced higher levels of clozapine- and scopolamine-appropriate responding (as reflected by the strong positive correlation). This interpretation, however, is complicated by two of the drugs tested in that study. The benzodiazepine chlordiazepoxide doesn't have any activity at muscarinic receptors, but produced partial substitution for both clozapine and scopolamine. Also, the tetracyclic antidepressant mianserin, which has some, but minimal, activity at muscarinic receptors, fully substituted for clozapine but not for scopolamine. Thus, while antagonism of muscarinic receptors is sufficient to elicit clozapine-appropriate responding, it is clear that other receptor mechanisms may play a role in clozapine's discriminative stimulus properties in rats. What has emerged as a result of these findings is the idea that clozapine's discriminative stimulus is mediated by a compound cue that involves antagonism of two or more receptors [31, 37, 48–54]. This idea is also supported by the fact that several

antipsychotic drugs (e.g. zotepine and quetiapine) substitute fully for clozapine even though they display a very low affinity for muscarinic receptors [48].

The idea of a compound cue for clozapine is also supported by the finding that training dose affects clozapine's discriminative stimulus. Porter et al. [53] trained rats to discriminate a low 1.25 mg/kg dose of clozapine instead of the 5 mg/kg dose that has been used in the majority of the studies. They found that the atypical antipsychotic drugs olanzapine, sertindole, and risperidone reliably substituted for clozapine. These antipsychotic drugs do not substitute for clozapine when the higher 5 mg/kg dose is used [49, 52]. Also, the atypical antipsychotic quetiapine produces full substitution for the 5 mg/kg clozapine cue [49, 52], but displayed only partial substitution for the 1.25 mg/kg clozapine cue [53]. While the basis for the 1.25 mg/kg clozapine discriminative cue is not known, unpublished observations from my lab have found that the muscarinic antagonist scopolamine does not substitute for 1.25 mg/kg clozapine; however, the somewhat selective serotonergic 5-HT_{2A} receptor antagonist MDL100907 [(*R*)-(+)-α-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-piperidinemethanol] engenders full clozapine-lever responding at a dose of 1.0 mg/kg. These findings offer further support for the role of multiple receptors in clozapine's discriminative stimulus properties (i.e., a compound cue) and also demonstrate that the nature of this cue depends (at least in part) on the training dose (for discussion of effects of training dose, see Chapters 1 and 3).

The nature of clozapine's discriminative stimulus properties also depends on the species. In pigeons trained to discriminate 1 mg/kg (i.m.) of clozapine, Hoenicke et al. [36] reported that antagonism of 5-$HT_{2A/2C}$ serotonergic receptors mediates clozapine's discriminative cue. This conclusion was based on the substitution of drugs (cyproheptadine, metergoline, mianserin, pizotifen, and fluperlapine) for clozapine that are antagonists at both 5-HT_{2A} and 5-HT_{2C} serotonergic receptors. This serotonergic mechanism was confirmed by Philibin et al. [33] in C57BL/6 mice trained to discriminate 2.5 mg/kg (s.c.) of clozapine from vehicle. The 5-$HT_{2A/2C}$ serotonergic antagonist ritanserin [55] fully substituted for the clozapine cue in C57BL/6 mice, in contrast to findings reported in rats by Wiley and Porter [56, 57]. Philibin et al. [33] also reported that the serotonergic agonist quipazine was able to block clozapine's discriminative stimulus in C57BL/6 mice without disruption of responding. However, serotonergic antagonism is not sufficient to elicit clozapine-appropriate responding in DBA/2 mice. Porter et al. [35] reported that ritanserin produced no substitution in DBA/2 mice trained to discriminate 2.5 mg/kg (s.c.) of clozapine from vehicle. Another difference between C57BL/6 and DBA/2 mice is that the α_1-adrenoceptor antagonist prazosin fully substitutes for clozapine in C57BL/6 mice but not in DBA/2 mice (for further discussion of strain differences, see Chapter 3). In summary, it is clear that the mechanisms that mediate clozapine's discriminative cue may represent a compound cue in which antagonism at one receptor may be sufficient to elicit the cue (e.g., muscarinic antagonism with scopolamine in rats), but may not be necessary as blockade at one or more different receptors may also elicit clozapine's cue. The mechanisms for clozapine's cue also vary across different training doses in rats and across different species of animals. Clearly, additional research will be required to fully delineate clozapine's discriminative stimulus properties.

E. BENZODIAZEPINE ANTAGONISTS

Flumazenil (Ro 15-1788) is a benzodiazepine antagonist that was initially characterized as not having any behavioral activity [58], but other studies have indicated that it can have weak benzodiazepine-like effects at high [59] or moderate [60] doses. This suggests that flumazenil may have partial agonist properties. De Vry and Slangen [61] trained rats to discriminate 10 mg/kg (i.p.) of flumazenil from saline after about 60 training sessions and found a dose-dependent, but flat generalization gradient for flumazenil (ED_{50} = 0.12 mg/kg), clearly demonstrating that flumazenil was capable of producing behavioral effects in rats. In a subsequent study, Woudenberg and Slangen [62] trained rats to discriminate a 15 mg/kg (i.p.) dose of flumazenil from saline, They also found that the flumazenil generalization curve had a very flat slope and the ED_{50} of 0.17 mg/kg was similar to that seen in the De Vry and Slangen study. Interestingly, the benzodiazepines chlordiazepoxide and diazepam (both considered to be agonists) fully substituted for flumazenil; whereas, other benzodiazepines (e.g., midazolam) produced only partial substitution. Also, a number of non benzodiazepine compounds produced partial substitution for flumazenil suggesting that the agonist properties of flumazenil may not be at the benzodiazepine receptor and that the discriminative stimulus for flumazenil has a different degree of specificity.

In contrast to the findings by Woudenberg and Slangen [62], Wong et al. [63] found that chlordiazepoxide and midazolam did not substitute for flumazenil in pigeons trained to discriminate 0.1 mg/kg (i.m.) of flumazenil from saline and based on a series of tests with selective ligands they concluded that the discriminative stimulus effects of flumazenil involve diazepam-insensitive $GABA_A$ receptors. Koek et al. [64] also reported that the benzodiazepine diazepam and the $GABA_A$ agonists muscimol and THIP failed to substitute for flumazenil in pigeons trained to discriminate 0.1 mg/kg (i.m.) of flumazenil. These studies with pigeons demonstrate a clear species difference with regard to the training dose needed to establish flumazenil as a discriminative stimulus (a 100-fold lower training dose in pigeons as compared to rats; although the route of administration might have an effect) and the ability of classical benzodiazepines like chlordiazepoxide and midazolam to substitute for flumazenil's discriminative stimulus in rats but not in pigeons. Flumazenil has also been established as a discriminative stimulus in humans [65]. Interestingly, midazolam occasioned a dose-dependent increase in flumazenil-appropriate responding in humans trained to discriminate 0.56 mg/70 kg (i.v.) of flumazenil form saline, reaching a maximum of 75% flumazenil-appropriate responding at the highest dose tested (1.0 kg/70 kg). Based on the results from this study, Smith and Bickel [65] concluded that flumazenil does not act as a traditional benzodiazepine agonist.

F. CANNABINOID ANTAGONISTS

While there has been extensive research [e.g., 66 and Chapter 8] on the discriminative stimulus properties of Δ^9-tetrahydrocannabinol (Δ^9-THC; believed to be the primary psychoactive ingredient of marijuana [*Cannabis sativa*]), studies on the discriminative

stimulus properties of cannabinoid CB_1 receptor antagonists were not possible until the synthesis of SR 141716A (rimonabant) in 1994 [67]. However, initial attempts to establish rimonabant as a discriminative stimulus in food-reinforced operant procedures were unsuccessful in both rats and pigeons [68, 69]. However, Järbe and his associates [70, 71] utilized a conditioned taste aversion (CTA) drug discrimination procedure [see 72] to study the discriminative stimulus properties of rimonabant in rats. The training dose for rimonabant was 5.6 mg/kg in the first study [70] and 5.6 or 3.0 mg/kg in the second study [71]. They found that the rimonabant analog AM251 (1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide) fully substituted for the rimonabant discriminative cue; whereas, the cannabinoid CB_2 antagonists SR 144528 (*N*-[(1S)-endo-1,3,3-trimethyl bicyclo [2.2.1] heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide) and AM630 (iodopravadoline), the cannabinoid CB_1 agonist methanandamide (mAEA), Δ^9-THC, flumazenil, naloxone, morphine, and d-amphetamine did not substitute for rimonabant. As expected, Δ^9-THC produced a dose-related attenuation of the rimonabant-induced suppression of saccharin drinking (i.e., the discriminative cue for rimonabant was blocked). The cannabinoid CB_1 agonist mAEA did not block rimonabant's discriminative cue. While the inability of mAEA to block the discriminative stimulus properties of rimonabant is inconsistent with the other findings in their studies, the authors suggested that antagonism of cannabinoid CB_1 receptors is an important factor for rimonabant's discriminative stimulus properties.

McMahon [73, 74] has shown that rimonabant can be established as a discriminative stimulus in rhesus monkeys pretreated with Δ^9-THC. In the first study [73], rhesus monkeys were treated twice daily with 0.56 mg/kg of Δ^9-THC and trained to discriminate 1 mg/kg of rimonabant from vehicle. They found that the generalization curve for rimonabant's discriminative stimulus was dose-related and could be attenuated by acute administration of Δ^9-THC. The dopamine agonist cocaine and the NMDA antagonist ketamine did not generate rimonabant-appropriate responding. Interestingly, when the daily administration of Δ^9-THC was discontinued, the animals displayed rimonabant-appropriate responding. The authors concluded that the discriminative stimulus properties of rimonabant appeared to be mediated by antagonism of cannabinoid receptors and that it might be related to symptoms of withdrawal from Δ^9-THC.

In the second study utilizing this procedure [74], a smaller dose of Δ^9-THC (0.32 mg/kg) was used to minimize the effects of Δ^9-THC on those days when Δ^9-THC was not administered prior to testing. Also, Δ^9-THC was administered only once each day, 30 minutes before sessions. The training dose of rimonabant was 1 mg/kg. McMahon found that the discriminative stimulus effects of rimonabant were more consistent than in the previous study [73] and confirmed that drug discrimination can be a useful procedure for studying cannabinoid dependence and withdrawal. Specifically, they found that an additional pre-session dose (0.32 mg/kg) of Δ^9-THC produced a 3-fold rightward shift in the rimonabant dose-response curve. The CB_1 antagonist AM 251 fully substituted for the discriminative cue of rimonabant; whereas, the NMDA antagonist ketamine and the benzodiazepine midazolam did not. They also found, as in the previous study [73], that omission of the presession dose of Δ^9-THC generated responding on the rimonabant-appropriate lever.

G. CHOLINERGIC ANTAGONISTS

One of the first two-lever drug discrimination studies ever conducted involved training the muscarinic receptor antagonist atropine [9]. Rats were trained to discriminate 10 mg/kg (i.p.) of atropine from saline. Both atropine (ED_{50} = 0.8 mg/kg) and scopolamine (ED_{50} = 0.06 mg/kg) produced a dose-dependent pattern of responding on the atropine lever. The atropine discriminative cue was centrally mediated as shown by the lack of atropine-appropriate responding produced by the quaternary compound atropine methyl bromide, which does not readily cross the blood-brain barrier.

As Jung et al. [75] point out, the doses used to study muscarinic antagonists as the training drug in the T-maze discrimination procedure have typically been very high [see 76, 77]. Jung et al. used a two-lever food-reinforced drug discrimination procedure to increase the sensitivity of scopolamine discrimination by initially training rats to discriminate 0.25 mg/kg (s.c.) of scopolamine from saline. The training dose was gradually reduced to 0.062 mg/kg and generalization testing with scopolamine yielded an ED_{50} value of 0.27 mg/kg. A time course analysis of the scopolamine discriminative cue revealed that 30 minutes (used for training) and 60 minute presession injection times resulted in full drug-lever selection. However, when the injection time was lengthened to 120 and 240 minutes, the rats shifted responding to the vehicle lever (<20% drug-lever responding at the 240 minute injection time). The scopolamine cue also was centrally mediated as scopolamine methylbromide (1 mg/kg), which does not readily cross the blood-brain barrier, produced only vehicle-lever responding. The muscarinic receptor antagonists atropine and trihexyphenidyl produced full substitution for the scopolamine discriminate cue, with ED_{50} values of 2.20 mg/kg and 0.21 mg/kg, respectively. They also conducted antagonism testing with several muscarinic agonists (arecoline, oxotremorine, physostigmine, and tetrahydroaminoacridine) trying to block the scopolamine cue, but obtained mixed results. Part of the problem they experienced was that as they increased the doses of the muscarinic agonists, response rate suppression increased dramatically and that precluded meaningful interpretation for much of the data with regard to blockade of the scopolamine cue. Oxotremorine significantly antagonized scopolamine's discriminative stimulus at the highest tested dose (0.30 mg/kg), but response rates were reduced to 8% of saline responding and no lever selection data was available for two rats whose responding was completely suppressed. Similar results were obtained with the acetylcholinesterase inhibitor tetrahydroaminoacridine at the highest dose of 10 mg/kg. The clearest results were obtained with physostigmine, which produced a significant dose-dependent antagonism of the scopolamine cue at four doses (0.10, 0.20, 0.40, and 0.80 mg/kg) with significant decreases in response rates only at the two highest doses. Because of the problems with antagonism of the scopolamine discriminative stimulus, Jung et al. suggested that the muscarinic agonist discriminative cue might be more useful for studying muscarinic receptor mechanisms.

In another study by Jung et al. [78], the underlying mechanisms of the scopolamine discriminative stimulus were explored by intracerebroventricular (i.c.v.) injections of pirenzepine, a relatively selective muscarinic m_1 receptor antagonist, which does not readily cross the blood-brain barrier [79]. In rats trained to discriminate 0.062 mg/kg

(s.c.) of scopolamine, pirenzepine (20–40 µg i.c.v.) did not engender any scopolamine responding, whereas centrally administered scopolamine (1.5–12 µg i.c.v.) generalized in a dose-dependent manner. In a different group of rats trained to discriminate 0.075 mg/kg (s.c.) of oxotremorine (a muscarinic agonist), scopolamine (12 µg i.c.v.), but not pirenzepine (20–40 µg i.c.v.) antagonized oxotremorine's discriminative cue. Based on these results, the authors concluded that the m_1 receptor did not play a prominent role in the discriminative stimulus properties of scopolamine or oxotremorine. However, Kelley and Porter [50] found that the relatively selective m_1 antagonist trihexyphenidyl fully substituted for scopolamine in rats trained to discriminate 0.125 mg/kg scopolamine from saline (as did Jung et al. [75]; see above), but the highly selective m_2 antagonist BIBN 90 (5,11-dihydro-8-chloro-11-[[4-[3-[(2,2-dimethyl-1-oxopentyl) ethylamino]propyl]-1-piperidinyl]acetyl]-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one) [80] did not substitute for scopolamine. Based on these findings and also because scopolamine has a higher affinity for m_1 receptors than for m_2 receptors [80], Kelley and Porter concluded that m_1 muscarinic receptor antagonism plays a greater role than m_2 receptor antagonism in scopolamine's discriminative stimulus. The differences between the Kelley and Porter [50] study and the Jung et al. [75] study may be due to several factors. Both the training dose and route of administration differed between the two studies (0.062 mg/kg, s.c. versus 0.125 mg/kg, i.p.), and the selective m_1 antagonist pirenzepine was administered i.c.v. in the Jung et al. study.

In another study, Kelly et al. [81] trained rats to discriminate 0.25 mg/kg (i.p.) of scopolamine from saline and compared scopolamine's discriminative stimulus to the discriminative stimulus for the serotonergic receptor antagonist mianserin (another group of rats was trained to discriminate 4 mg/kg (i.p.) of mianserin from saline). Surprisingly, an asymmetrical cross-generalization between these two drugs was found. The muscarinic antagonist scopolamine fully substituted for the serotonergic antagonist mianserin, but mianserin did not engender any scopolamine-appropriate responding (no cross-generalization between these two drugs had been expected). Given that mianserin has only minimal activity at muscarinic receptors [82, 83], it is not clear why scopolamine substituted for mianserin's discriminative cue.

H. DOPAMINE ANTAGONISTS

While a large number of drug discrimination studies have been conducted with dopamine D_1 and D_2 receptor agonists [e.g., see 84], to date, only four drugs characterized as dopamine receptor antagonists have been established as discriminative stimuli. Two of them, chlorpromazine and haloperidol, are termed typical (i.e., first generation) antipsychotics that have high binding affinities for dopamine D_2 receptors, whereas haloperidol, though relatively selective for D_2 receptors (K_i = 1.4 nM), also displays a fairly high affinity for serotonin 5-HT_{2A} receptors (25 nM, K_i) and α_1-adrenoceptors (19 nM, K_i). Chlorpromazine has a high affinity at D_2 receptors (1.2 nM, K_i), serotonin 5-HT_{2A} receptors (K_i = 3.3 nM), α_1-adrenoceptors (K_i = 14 nM,), and histamine H_1 receptors (K_D = 9 nM,) [25, 41, 55, 85–88]. The third dopamine antagonist is tiapride, a selective dopamine D_2/D_3 receptor antagonist [89]. The fourth dopamine antagonist is PNU-99194A, a relatively selective dopamine D3 receptor antagonist [159, 160].

1. Chlorpromazine

Chlorpromazine was the first dopamine receptor antagonist to be established as a discriminative stimulus. Stewart [90] trained rats to discriminate 4.0 mg/kg (i.p.) of chlorpromazine from saline in a shock-avoidance task and reported that the chlorpromazine discriminative stimulus generalized to several phenothiazines that were tested. Overton [91] was unable to establish a 5.0 mg/kg (i.p.) dose of chlorpromazine in a shock-avoidance T-maze task in rats. Also, Harris and Balster [10] could not establish discriminative control with a 1.0 mg/kg dose of chlorpromazine in a two-lever drug discrimination procedure with rats. Barry et al. [92] were the first to successfully establish drug discrimination with 1.0 mg/kg (i.p.) dose of chlorpromazine in a two-lever operant task (food reinforcement on one lever; shock punishment on the other lever) in rats. They reported that chlorpromazine's discriminative cue was mediated centrally, as a peripherally acting quaternary form of chlorpromazine failed to substitute for chlorpromazine.

Goas and Boston [93] established a two-lever discrimination between 2.0 mg/kg of chlorpromazine (p.o.) versus vehicle in rats and reported that haloperidol, clozapine, and the muscarinic receptor antagonist benztropine mesylate produced full substitution for chlorpromazine, whereas another CNS depressant, chlordiazepoxide, did not. They also were able to establish a drug-drug discrimination with 8.8 mg/kg clozapine versus 4.24 mg/kg of chlorpromazine (p.o.). Haloperidol generated chlorpromazine-appropriate responding at doses of 1.0 and 2.0 mg/kg, but produced clozapine-appropriate responding at 0.5 mg/kg. Also, it should be noted that chlorpromazine did not substitute for clozapine in rats trained to discriminate 6.9 mg/kg (p.o.) of clozapine from vehicle. Thus, the generalization between clozapine and chlorpromazine was not symmetrical.

Porter et al. [94] were able to further explore the relationship between chlorpromazine's discriminative cue and other antipsychotic drugs in rats trained to discriminate 1 mg/kg (i.p.) of chlorpromazine from vehicle in rats. They reported that the atypical antipsychotics clozapine and olanzapine and the typical antipsychotic thioridazine fully substituted for chlorpromazine. Unlike the findings in the Goas and Boston [93] study, haloperidol produced only partial substitution for chlorpromazine; however, it should be noted that there were differences in the route of administration and dose of the training drug that may account for this difference. In a subsequent study, Porter et al. [95] used a three-choice drug discrimination between 5 mg/kg of clozapine versus vehicle versus 1 mg/kg of chlorpromazine in rats. While there was not complete cross-generalization between clozapine and chlorpromazine, a 4 mg/kg dose of chlorpromazine produced partial substitution for clozapine (a 0.3125 mg/kg dose of clozapine substituted for chlorpromazine); this was contrary to what Goas and Boston [93] reported in their study (i.e., no substitution for clozapine). The atypical antipsychotic risperidone and the typical antipsychotic haloperidol fully substituted for chlorpromazine, but not for clozapine. The atypical antipsychotic olanzapine produced partial substitution for both clozapine and chlorpromazine. Testing with selective ligands revealed that only the α_1-adrenoceptor antagonist prazosin produced full substitution for the chlorpromazine discriminative stimulus. The muscarinic antagonist scopolamine generated partial substitution on both the clozapine- and chlorpromazine-appropriate levers. The results of these studies suggest that there is some overlap in the discriminative stimulus properties of typical and atypical antipsychotic drugs.

2. Haloperidol

Haloperidol has been more difficult to establish as a discriminative stimulus in the standard two-lever drug discrimination paradigm. In an initial report Colpaert et al. [96] trained rats to discriminate 0.02 mg/kg (s.c.) of haloperidol from saline. However, it required over 80 training sessions to establish the discrimination and no drugs were tested for substitution. McElroy et al. [97] also were able to establish haloperidol (0.05 mg/kg, i.p.) as a discriminative stimulus in rats in a mean of 45 training sessions—much fewer than in the Colpaert et al. [96] study. Haloperidol's generalization curve was dose-dependent with an ED_{50} = 0.008 mg/kg and the antipsychotic chlorpromazine fully substituted for haloperidol. Thus, chlorpromazine and haloperidol cross-generalize to each other's discriminative stimulus [see 93], which suggests that they may share a similar in vivo mechanism of action—that is, antagonism of dopamine D_2 receptors [see 16, 98]. The suggestion that dopamine antagonism mediates the discriminative stimulus properties of haloperidol and chlorpromazine is also supported by McElroy and co-workers' finding that the indirect dopaminergic agonists amphetamine (1 mg/kg) and cocaine (10 mg/kg) blocked haloperidol's discriminative cue without any suppression of response rates. It would be interesting to confirm this possibility by determining whether dopamine D_2 agonists could block the discriminative stimulus properties of haloperidol and chlorpromazine.

Haloperidol also has been established as a discriminative stimulus in drug-drug discrimination [56] and in three-choice drug discrimination studies. In an interesting amphetamine-haloperidol discrimination, Haenlein et al. [99] trained rats to discriminate one of three doses of d-amphetamine (0.1, 0.3, or 0.5 mg/kg) or vehicle versus haloperidol (0.02 mg/kg). The authors argued that this procedure allowed the assessment of a continuum of dopamine mediated cues. In support of this argument they found that rate of acquisition of the discrimination was dependent on the training dose for amphetamine. As the dose of amphetamine was increased, the rate of acquisition was also increased. The authors interpreted this as reflecting an increase in the net difference between the cue saliency of amphetamine and haloperidol. They also found that, following chronic dosing with amphetamine, the rats responded during drug-free testing as if they had received haloperidol and, conversely, drug-free testing following chronic haloperidol produced amphetamine-appropriate responding. This amphetamine-haloperidol drug discrimination also has been used successfully by Barrett et al. [100, 101] to gain better understanding of tolerance and withdrawal effects following treatment with amphetamine [100] and with nicotine [101].

This approach of pairing the dopamine agonist amphetamine (or cocaine) with the dopamine antagonist haloperidol has been expanded to a three-choice drug discrimination (a vehicle choice was added) in a number of studies [100, 102–106]. Using this three-choice procedure (amphetamine versus vehicle versus haloperidol), Barrett et al. [107] continued to explore withdrawal, tolerance, and sensitization to dopamine-mediated cues. They found that tolerance to amphetamine and haloperidol resulted in baseline shifts in the discriminative cues of these drugs rather than a reduction. They suggested that this three-choice procedure provides a good model for studying the aversive consequences (e.g., anhedonia and dysphoria) of amphetamine withdrawal

[see also 102, 103]. This three-choice model has also been used to characterize withdrawal effects with cocaine [104, 105] with very similar results. These studies support the utility of both two-choice and three-choice drug discrimination models for gaining a better understanding of drug withdrawal effects (rebound effects) and how this may be related to a specific neural mechanism (i.e., dopamine receptors in these studies).

3. Tiapride

Cohen et al. [108] were able to successfully train the D_2/D_3 dopamine receptor antagonist tiapride, a benzamide derivative with dopamine antagonist actions similar to sulpiride, in rats in a standard two-lever food reinforcement drug discrimination procedure. A large number of drugs was tested for substitution and for the ability to block the discriminative stimulus cue of tiapride. All of the drugs tested in this study (amisulpride, sulpiride, sultopride, clebopride, raclopride, metoclopramide, remoxipride, pimozide, thioridazine, olanzapine, chlorpromazine, risperidone, and haloperidol) with dopaminergic antagonist activity (both benzamides and nonbenzamide derivatives) substituted for tiapride with the notable exception of the atypical antipsychotic drug clozapine. Drugs that did not have dopamine antagonist activity did not substitute for tiapride. Finally, Cohen et al. were able to show that the discriminative stimulus properties of tiapride were mediated by dopamine D_2/D_3 receptors but not by D_1 receptors. The direct D_2/D_3 receptor agonists, quinpirole and 7-OH-DPAT, were able to significantly attenuate the discriminative stimulus effects of tiapride; whereas, the D_1 agonist SKF 38393 was not. The indirect DA agonist amphetamine was able to completely block tiapride's discriminative cue.

4. PNU-99194A

The fourth dopamine antagonist that has been established as the training drug in two-lever drug discrimination with rats is PNU-99194A, a relatively selective dopamine D_3 antagonist [159, 160]. Baker et al. [159] trained rats to discriminate 10.0 mg/kg (sc) PNU-99194A from saline and found that the psychomotor stimulants cocaine, amphetamine, and caffeine did not substitute for PNU-99194A. In a second study, Franklin et al. [160] reported that haloperidol (non-selective dopamine D_2 antagonist) and the mixed D_2/D_3 antagonists amisulpride and sulpiride also failed to substitute for PNU-99194A. However, the D_3-preferring antagonists (−)-DS121 and (+)-AJ76 fully substituted for PNU-99194A. Based on the results from these two studies, the authors concluded that the discriminative stimulus for PNU-99194A appears to be based on D_3 receptor antagonism and that the discriminative cue produced by PNU-99194A is not similar to that of psychostimulants.

I. GABAERGIC ANTAGONISTS

Pentylenetetrazol (PTZ) is a $GABA_A$ (γ-aminobutyric $acid_A$) receptor antagonist that has been studied extensively in preclinical models of anxiety. In an excellent review

article, Jung et al. [109] examined the discriminative stimulus effects of PTZ as a model of anxiety and provided a comprehensive review of this literature. To briefly summarize their findings, they concluded that PTZ drug discrimination is a good model of $GABA_A$ mediated anxiety. Evidence for this conclusion includes the fact that anxiogenic drugs that antagonize $GABA_A$ receptors produce PTZ-appropriate responding (i.e., substitute for PTZ's discriminative cue) and that anxiolytic drugs that are $GABA_A$ receptor agonists (e.g., midazolam) block the PTZ cue. Interestingly, anxiolytic drugs that do not work via $GABA_A$ mechanisms (e.g., buspirone) have little or no effect on the discriminative stimulus properties of PTZ. Thus, while PTZ's discriminative cue appears to be primarily mediated by antagonism of $GABA_A$ receptors, it can be modulated by a variety of non-GABA drugs, including 5-$HT_{1B/2C}$ agonists, 5-HT_3 antagonists, L-type calcium channel blockers, nicotine, NMDA, and strychnine. The role of nitric oxide synthase (NOS) inhibitors in the discriminative stimulus properties of PTZ has also been examined by Uzbay and Lal [110]. They found that NOS inhibitors ($_L$-NAME [$_L$-N^G-nitro arginine methyl ester], 7-NI [7-nitroindazole], and agmatine) did not substitute for PTZ's discriminative stimulus and that these compounds did not block PTZ's cue. Thus, nitric oxide does not appear to play any role in the discriminative stimulus properties of PTZ.

J. OPIATE ANTAGONISTS

While there have been many studies examining the discriminative stimulus properties of opiate agonists, examination of the discriminative stimulus properties of opiate antagonists has been limited to a relative small number of studies and drugs. One of the issues that appeared early in the literature was that it is very difficult to establish opioid antagonists as discriminative stimuli unless the animals were opioid-dependent [see 111, 112]. The majority of these studies have looked at the opiate antagonists naloxone and naltrexone, but the discriminative stimulus properties of diprenorphine also have been studied. The discriminative stimulus properties of the mixed opiate agonist-antagonists pentazocine, cyclazocine, and nalorphine have been examined in two studies.

1. Naloxone

It has been shown in a number of studies that the opiate antagonist naloxone can be trained as a discriminative cue in both rats [111–114] and pigeons [115]; however, fairly high training doses and extensive training have usually been required. Also, Lal et al. [111] reported that initial attempts to establish 10 mg/kg of naloxone as a discriminative stimulus was not successful in morphine-naïve rats, so a daily dosing regimen was implemented in which the rats were injected with morphine (40 mg/kg, i.p.) 8 hours prior to the naloxone or vehicle injections for the drug discrimination testing sessions. After successfully establishing the naloxone discrimination (approximately 50 training sessions), testing with the opiate agonist morphine and the mixed agonist-antagonist cyclazocine revealed no substitution for the naloxone discriminative cue. Similarly,

naloxone did not substitute for morphine or cyclazocine in rats trained to discriminate those drugs.

Weissman [116] also found that successful discrimination of naloxone was dependent on the physiological state of the organism. He established two training groups: in Group One the rats were trained to discriminate 3.2 mg/kg of naloxone versus vehicle; in Group Two the rats were trained to discriminate 3.2 mg/kg of naloxone + 5 mg/kg of morphine versus vehicle + 5 mg/kg of morphine (all injections were i.p. one hour prior to testing). After 56 discrimination training sessions, the rats in Group One averaged only 50–60% correct lever selection, whereas the rats in Group Two displayed over 80% correct lever selection for the training sessions 35 to 56. Weissman discussed the possibility that these results could be interpreted as simply a discrimination between a "morphine cue" (the vehicle + morphine group) versus a "no morphine cue" (the naloxone + morphine group) as naloxone antagonizes the discriminative stimulus effects of morphine [e.g., 117, 118]; however, it should be noted that this was not the case in the Lal et al. [111] study (morphine was not co-administered with the training drug and vehicle). Finally, in a somewhat analogous situation, Weissman [116] had previously demonstrated the importance of the physiological state of the animals in trying to establish drug discrimination with aspirin. He found that arthritic rats acquired the aspirin versus saline discrimination more readily than non-arthritic rats.

Given the difficulty of establishing naloxone discrimination in non-opiate dependent animals in the two previous studies, Carter and Leander [115] noted that part of the problem might be that the doses of naloxone used in the Lal et al. [111] and the Weissman [116] studies were too low (10 mg/kg and 3.2 mg/kg, respectively). Therefore, they increased the training dose of naloxone to 30 mg/kg (i.m.) and they also used pigeons instead of rats. While it took extended training to establish the discrimination with a mean of 79 sessions, near 100% correct responding was observed in non opiate-dependent pigeons. Naltrexone, pentazocine, levallorphan, and nalorphine produced nearly 100% correct naloxone-appropriate responding, whereas the opiate agonist morphine generated vehicle-appropriate responding. The underlying mechanisms responsible for naloxone's discriminative cue in pigeons remain unclear as diprenorphine did not generalize to the naloxone cue. Also, as the authors suggested, the 30 mg/kg dose might be affecting mechanisms other than blockade of just opiate receptors as naloxone is an effective opiate antagonist in pigeons in very low doses (0.3 mg/kg; see 119]. Since both the training dose and species differed in this study, it is difficult to directly compare this study to the first two studies with rats and lower training doses. However, all three of these studies clearly illustrate the difficulties associated with establishing naloxone as a discriminative stimulus and the importance of training dose (and perhaps species).

The discriminative stimulus properties of naloxone also have been studied in a series of studies by Riley and his colleagues (see review by Riley [7]) using a conditioned taste aversion (CTA) drug discrimination procedure [120–122]. In the initial study, Kautz et al. [120] administered 1.0 mg/kg of naloxone to rats prior to a saccharin-LiCl pairing and vehicle was administered prior to saccharin alone. After only three conditioning trials the discrimination was established as the rats avoided saccharin when injected with naloxone, but readily drank the saccharin solution when injected

with vehicle. The naloxone discrimination was dose dependent with rats displaying increased naloxone-appropriate responding as the naloxone dose was increased. The opiate antagonist naltrexone produced dose-dependent naloxone-appropriate responding; whereas, the opiate agonist morphine produced only vehicle-appropriate responding at all of the tested doses. In the second study, Smurthwaite et al. [121] reported that a series of opiate antagonists (naltrexone, diprenorphine, and nalorphine) with varying affinity for the mu opiate receptor all produced full or partial substitution for the naloxone discriminative cue.

On the basis of the results from these two studies, they concluded that naloxone's discriminative stimulus properties appeared to be mediated by mu opiate receptors. The third study [122] explored the role of the kappa and delta opiate receptors in naloxone's discriminative stimulus properties as naloxone also has affinity for these opiate receptors [123]. They tested compounds that had relatively selective affinity for the three different opiate receptors: naltrexone (mu receptors), naltrindole (delta receptors), and MR2266 (kappa receptors). They found that only naloxone (the discriminative stimulus) and naltrexone produced naltrexone-appropriate responding. Thus, naloxone's discriminative stimulus effects appear to be mediated via mu receptors and not at delta or kappa receptors. They were also able to demonstrate that naloxone's discriminative cue was centrally mediated using naltrexone methobromide, which does not readily cross the blood-brain barrier. This series of studies demonstrates the utility of the CTA drug discrimination procedure for studying the discriminative stimulus properties of a drug (i.e., naloxone) that is problematic in the standard food-reinforced two-level drug discrimination procedure. As discussed above, it has been necessary to use high doses and extensive training to establish naloxone as a discriminative stimulus in morphine-naïve animals [115] and other studies [111, 112] had to establish opiate-dependent animals in order to successfully establish the naloxone discrimination.

2. Naltrexone

Like naloxone, the opiate antagonist naltrexone has been established as a discriminative stimulus in opiate-dependent rats [124] in opiate-dependent pigeons [125–127] and in opiate-dependent rhesus monkeys [e.g., 128, 129]. France and Woods [125] trained pigeons in a three-choice drug discrimination with morphine (17.3 mg/kg) versus vehicle versus naltrexone (0.032 mg/kg). The pigeons received a 10 mg/kg morphine injection 6 hours prior to test sessions. They found that the opiate receptor antagonists naloxone, nalmefene, and diprenorphine fully substituted for naltrexone. The mixed agonist-antagonist nalorphine substituted for naltrexone at higher doses (>10 mg/kg); whereas, the mixed agonist/antagonist pentazocine failed to substitute for either naltrexone or morphine. When administered concomitantly, morphine and naltrexone were able to attenuate their respective discriminative cues. The authors concluded that these findings suggested that opposing actions at the same receptors were responsible for mediating the discriminative stimulus properties of naltrexone and morphine. France and Woods [127] also have established a two-choice discrimination between morphine (5.6 mg/kg, i.m.) and naltrexone (10 mg/kg) in nondependent pigeons. They reported that a single injection of 10 mg/kg and 32 mg/kg morphine 24 hours prior to the admin-

istration of naltrexone resulted in 3- and 10-fold left-ward shifts (i.e., increased sensitivity to naltrexone's discriminative stimulus). Based on these results they concluded that acute sensitivity to morphine differs from that observed following chronic morphine treatment.

In rhesus monkeys treated with daily injections of morphine (3 hours before testing with 1.78 or 3.2 mg/kg), two-lever drug discrimination with 0.01 mg/kg naltrexone versus saline was established [129]. The naltrexone generalization curve was dose dependent and as in previous studies they found that mu opiate antagonists (e.g., naloxone, nalorphine, and quadazocine) substituted for naltrexone; whereas, mu opiate agonists (e.g., morphine, U-50,488, and butorphanol) and nonopiate drugs (e.g., ketamine and pentobarbital) did not substitute for naltrexone. As expected, quaternary naltrexone did not substitute for naltrexone, indicating that naltrexone's discriminative stimulus was centrally mediated in rhesus monkeys as in other species.

While the previously discussed studies have indicated that the discriminative stimulus properties of naloxone and naltrexone appear to be mediated by mu opiate receptors, Sell and France [128] reported that the dopamine agonists cocaine and amphetamine attenuated naltrexone's discriminative cue in opiate-dependent rhesus monkeys. Both cocaine and amphetamine produced significant right-ward shifts in naltrexone's dose-effect curve. Interestingly, haloperidol (dopamine antagonist) produced a left-ward shift in naltrexone's dose-effect curve. Sell and France concluded that these results implicated a role for multiple neurotransmitter systems during opiate withdrawal and were consistent with other findings that dopamine levels decrease during opiate withdrawal.

3. Diprenorphine

The discriminative stimulus properties of the nonselective opiate receptor antagonist diprenorphine [130, 131] have been studied both in a two-choice drug discrimination procedure in squirrel monkeys [132] and in the CTA drug discrimination paradigm in rats [133]. DeRossett and Holtzman [132] trained squirrel monkeys to discriminate 0.1 mg/kg diprenorphine (i.m.) from vehicle in a two-choice procedure using a discrete-trial shock avoidance procedure. As seen in studies with nondependent animals with naloxone or naltrexone it required a lengthy training regimen to establish diprenorphine as a discriminative stimulus (mean of 118 training sessions). However, once the discrimination was established it remained relatively stable. A generalization dose-effect curve for diprenorphine yielded an $ED_{50} = 0.004$ mg/kg. The most interesting finding in this study was that the selective opiate antagonist naloxone, naltrexone, and WIN 44,441-3 (2α,6α,11S*)-(-)-1-cyclopentyl-5-(1,2,3,4,5,6-hexahydro-8-hydroxy-3,6,11-trimethyl-2,6-methano-3-benzazocin-11-yl)-3-pentanone methane sulfonate) failed to substitute for diprenorphine. This suggested that the discriminative stimulus properties of diprenorphine were NOT mediated by antagonism of mu or kappa receptors.

Using the CTA drug discrimination procedure, Smurthwaite and Riley [133], reported that the opiate antagonists naloxone and naltrexone and the mixed agonist/antagonist nalorphine all produced dose-dependent substitution for diprenorphine. In contrast, the opiate agonist morphine and barbiturate pentobarbital (nonopiate) did not substitute for diprenorphine. These results were in direct contrast to those reported by

DeRossett and Holtzman [132]. As discussed by Smurthwaite and Riley, there were differences between these two studies including the species (rats versus squirrel monkeys) and procedural differences (CTA procedure versus discrete trial shock-avoidance procedure). Smurthwaite and Riley suggested that the CTA drug discrimination procedure appears to be a more sensitive assay for establishing discriminative stimuli with opiate antagonists in opiate-naïve animals. This increased sensitivity is reflected by the dose, speed of acquisition, and the resulting generalization patterns seen in this drug discrimination procedure.

4. Nalorphine

Hirschhorn [134] established the mixed opiate agonist/antagonists nalorphine (*N*-allyl-normorphine, a mu receptor antagonist and kappa receptor agonist), pentazocine, and cyclazocine as discriminative stimuli in separate groups of rats. The opiate antagonist naloxone blocked the discriminative cue for each of these drugs. Interestingly, while pentazocine and cyclazocine substituted for nalorphine, nalorphine did not substitute for either pentazocine or cyclazocine. Thus, cross-generalization between these three mixed agonists/antagonists was not symmetrical and the underlying mechanisms responsible for the discriminative stimulus properties of these drugs differed.

Nalorphine also has been studied in the CTA drug discrimination assay. Smurthwaite and Riley [135] reported that rats readily acquired the discrimination between 10 mg/kg (i.p.) of nalorphine and vehicle. While the mu opiate agonist morphine produced a dose-dependent substitution for nalorphine, the kappa opiate agonist U50,488H ((trans-(±)-3,4-dichloro-*N*-methyl-N-[2-(1,-pyrolidinyl)-cyclohexyl]benzeneacetamide)) and the mu opiate antagonists naloxone and naltrexone did not substitute for nalorphine. These findings indicated that the discriminative stimulus properties of nalorphine were mediated by agonist activity at mu receptors.

K. SEROTONERGIC ANTAGONISTS

1. Mianserin

Meert and Janssen [136] reported that they were unsuccessful in training rats to discriminate the serotonergic 5-HT_2 receptor antagonist ritanserin in a two-lever drug discrimination procedure. They tried a dose of 1.25 mg/kg (i.p.) for over 80 training sessions and a dose of 10 mg/kg (s.c.) for over 60 training sessions with no success. Kelley et al [81] were able to establish the serotonergic antagonist mianserin (a second generation tetracyclic antidepressant [137] as the training drug in rats trained to discriminate 4 mg/kg (i.p.) of mianserin from saline. The rats met the training criteria in a mean of 28 sessions (ED_{50} = 0.50 mg/kg). As mentioned above in the section on cholinergic antagonists, the muscarinic antagonist scopolamine fully substituted for mianserin with 88% drug lever responding at a dose of 1 mg/kg (response rates were significantly reduced at this dose), while mianserin did not substitute for scopolamine. The basis for this asymmetrical cross-generalization between mianserin and scopolamine is unclear, as mianserin has only minimal activity at muscarinic receptors [82,

83] and scopolamine is a nonselective muscarinic antagonist that has no activity at serotonergic receptors [138].

2. Pizotifen

Pizotifen (BC105) has traditionally been considered to be a serotonergic receptor antagonist and studies have shown that it can block the discriminative stimulus effects (presumably mediated by serotonergic receptor agonist activity) of hallucinogens (e.g., LSD [13]). However, Minnema et al. [139] found that a training dose of 6 mg/kg pizotifen (i.p., rats) did not generalize to a number of putative serotonergic antagonists, including methiothepin, xylamidine, and cinanserin. Interestingly, pizotifen generalized fully to cyproheptadine, which in addition to having serotonergic antagonist activity is a histamine H_1 receptor antagonist [138] and to the phenothiazine antihistamine promethazine. At lower training doses (1.0 and 3.0 mg/kg), they were not able to train rats to discriminate pizotifen from vehicle. Thus, the higher training dose (6.0 mg/kg) used in this study may have exerted its discriminative stimulus control over responding primarily through antihistaminergic activity rather than serotonergic antagonist activity. In studies using pizotifen to antagonize the discriminative stimulus effects of hallucinogens, lower doses (<3 mg/kg) have typically been used. These differences between low and high doses of pizotifen reinforce the notion that drug dose is a critical variable in drug discrimination studies (as well as in other behavioral studies). Different receptor mechanisms may be activated or predominate as the dose of a drug is increased or decreased (see Chapter 3). The importance of training dose has also been shown in drug discrimination studies with antipsychotic drugs [e.g., 53; also, see section on antipsychotic drugs for further discussion on this issue].

3. MDL 100907

Because of interest in the role of serotonergic mechanisms involved in the treatment of schizophrenia (specifically antagonism of serotonin receptors; see 140, 141], the ability to train animals to discriminate a serotonergic antagonist would be very useful in development of pharmacotherapies for schizophrenia. Dekeyne and Millan [142] successfully trained rats to discriminate the serotonergic 5-HT_{2A} receptor antagonist MDL100907 (0.16 mg/kg, i.p.) and demonstrated that its discriminative stimulus properties were mediated by antagonism of 5-HT_{2A} receptors but not by antagonism of 5-HT_{2B} or 5-HT_{2C} receptors. In a subsequent study [143] they compared the discriminative stimulus properties of MDL100907 to number of antipsychotic drugs and to other selective ligands. They found that MDL100907's discriminative cue dose-dependently generalized to a number of atypical antipsychotic drugs including clozapine, quetiapine, risperidone, and ziprasidone. All of these antipsychotic drugs display a preferential affinity to 5-HT_{2A} receptors as compared to dopamine D_2 receptors. Interestingly, the typical antipsychotic haloperidol which possesses greater affinity for D_2 receptors relative to 5-HT_{2A} receptors, also substituted for MDL100907. Since all of these drugs have antagonist activity at α_1-adrenoceptors, Dekeyne et al. tested several α_1-adrenoceptor drugs and they found that prazosin and WB4101 both fully substituted for MDL100907's discriminative cue. These results demonstrated that antagonism at

both 5-HT_{2A} receptors and α_1-adrenoceptors is sufficient for generalization to the discriminative stimulus properties of MDL100907.

4. WAY 100635

WAY 100635 has typically been used as a selective serotonergic 5-HT_{1A} receptor antagonist and has been characterized as having much higher selectivity for 5-HT_{1A} receptors relative to dopamine D_4 receptors [144]; although, Chemel et al. [145] have shown that WAY 100635 is a very potent and efficacious dopamine D_4 agonist. Because of this discrepancy regarding the in vitro properties of WAY 100635, Marona-Lewicka and Nichols [146] examined the role of 5-HT_{1A} and D_4 receptors in the behavioral in vivo effects of WAY 100635. After training rats to discriminate 10 μmol/kg (i.p.) of WAY 100635 versus saline (after initial attempts to train a dose of 0.74 μmol/kg were unsuccessful), they found that the selective 5-HT_{1A} agonists 8-OH DPAT and LY 293284 ((4*R*)-6-acetyl-4-(di-*n*-propylamino)-1,3,4,5-tetrahydrobenz[c,d]indole) did not block WAY 100635's discriminative stimulus. However, the dopamine D_4 antagonists sonepiprazole and A-381393 (2-[4-(3,4-Dimethylphenlyl)piperazin-1-ylmethyl]-1H benzoimidazole) dose-dependently attenuated WAY 100635's discriminative stimulus. Based on these results, Marona-Lewicka and Nichols concluded that the discriminative stimulus properties of WAY 100635 are mediated by activation of dopamine D_4 receptors—not by blocking 5-HT_{1A} receptors. Thus, these in vivo data support the in vitro findings of Chemel et al. [145] that WAY 100635 is a potent agonist at dopamine D_4 receptors.

L. SUMMARY

Receptor antagonists are often thought of as being inactive at receptors, that is, they have either no or very low efficacy. An interesting question was posed to me by Dr. John Rosecrans (personal communication)—if this is true, then how can receptor antagonists be established as training drugs in drug discrimination studies? I think there are several possibilities. One possibility is that some of these drugs may actually have agonist properties that form the basis of their discriminative stimulus. Another possibility is that many of these drugs are not select ligands. They also bind to other receptors and may have agonist or inverse agonist properties that may serve as a discriminative stimulus. Support for the idea that the discriminative stimulus properties of antihistamines may not depend entirely on antagonist activity at H_1 histamine receptors comes from studies in which several antihistamines have been found to substitute for the dopamine agonists d-amphetamine [28] and cocaine [29], and cocaine and amphetamine fully substituted for the over-the-counter drug mixtures, dextromethorphan + ephedrine and dextromethorphan + diphenhydramine [30]. Thus, it appears that the discriminative stimulus properties of antihistamine do not totally depend on antagonism of H_1 histamine receptors.

There were several other important factors that emerged from the literature of the discriminative stimulus properties of many of these receptor antagonists—these

included training dose, the species used in the study, and the type of drug discrimination procedure used. Thus, establishing the discriminative stimulus properties of receptor antagonists faces many of the same issues as typically seen with receptor agonists with perhaps greater difficulty for certain drug classes. However, as many of the studies discussed have shown, it is often possible to work around these problems by considering training issues such as dose, species, and different drug discrimination procedures. Drug discrimination is a powerful in vivo assay for determining the subjective effects of drugs (both agonist and antagonists) and to study the receptor mechanisms that mediate a drug's discriminative stimulus (and perhaps therapeutic effects).

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10

THE DISCRIMINATION OF DRUG MIXTURES[1]

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[1]This chapter is dedicated to Dr. Donald A. Overton in recognition of his seminal role in drug discrimination research and the invaluable guidance and support that he has given to the author.

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

A. INTRODUCTION

Most drugs are complex in the sense that they have multiple subjective effects and act through several neurobiological mechanisms. The term "discriminative stimulus complex" recognizes the potential for drug stimuli to be compound in nature [1]. Experimental psychopharmacology has as one of its aims the elucidation and separation of such effects and mechanisms. To achieve this goal, researchers often dream of working with model systems that reflect only a single effect or action, and because this is rarely possible, models are regarded as unsatisfactory. The work on which this review focuses turns this concept upon its head. It accepts the multiple sensitivities of model systems as an unavoidable fact of life, and tries to define empirically how different aspects of drug action interact to produce observed discriminative stimulus effects. This analysis is possible because of a functional similarity between drug-produced interoceptive stimuli and conventional exteroceptive stimuli that act on the auditory and visual systems, among others. The rules that govern compound exteroceptive discriminations may also apply to drug discriminations, and through them we have access to a theoretical foundation for analyzing complex drug-produced interoceptive cues. A purported weakness of drug stimuli as endpoints that is sometimes highlighted by workers using other techniques is their complexity. A more positive approach is to recognize this complexity as an asset that enables multiple aspects of drug action to be analyzed. All techniques are subject to confounds associated with the complexity of drug action, but drug discrimination, perhaps uniquely, has a theoretical basis for exploring these multiple actions.

Ideas on how associative processes could lead to interactions between drugs and exteroceptive stimuli, and between one drug with another, were first discussed in a conference report by Järbe et al. [2]. Such analysis requires that the drugs in the mixture produce separate interoceptive cues that do not overlap (i.e., generalize) with each other and that neither drug potentiates or antagonizes the pharmacological actions of the

other. The way in which different components of drug action interact to produce compound discriminative stimuli was therefore investigated by "synthesizing" complex drug cues with known component elements; this synthesis was achieved by administering mixtures of pharmacologically different substances to experimental animals and analyzing the characteristics of the resulting discriminative stimuli. Systematic studies using drug mixtures began with an investigation of mixtures of nicotine plus the short-acting benzodiazepine, midazolam [3].

As commonly occurs in science, there were precedents, as illustrated by isolated studies of the discrimination of drug mixtures that did not develop into a coherent body of work. Thus, Overton [4] found that a mixture of atropine and pentobarbital could function as a discriminative stimulus but did not analyze the effect further. Järbe and Johansson [5] studied discrimination of a mixture of the anticholinesterase physostigmine with the anticholinergic drug Ditran (the latter itself being a mixture), whereas Witkin et al. [6] reported briefly on discrimination of an amphetamine-barbiturate mixture. Snoddy and Tessel [7] examined discrimination between vehicle and a mixture of amphetamine plus nisoxetine, two drugs that generalized to each other in subjects trained on either drug alone. Studies of racemic forms of some drugs may also be seen as constituting training with mixtures. For example, studies of discriminative effects of 3,4-methylenedioxyamphetamine were interpreted as evidence that the (+)-isomer acted mainly on dopaminergic mechanisms whereas the (–)-isomer acted through 5-hydroxytryptamine receptors [8]. A direct intellectual precedent for the subsequent cohort of studies on the discrimination of drug mixtures can also be found in an abstract [9] describing the discrimination of a mixture of amphetamine plus lysergic acid diethylamide (LSD). In the other studies cited above, the drug mixtures used for training discriminations were chosen on the basis of some likely interactions between their component drugs rather than for the analysis of how separate drug actions combine to produce a perceived compound stimulus.

A key study by Holloway et al. [10] was the inverse of those cited above, in the sense that they demonstrated that rats trained on amphetamine generalized to abused mixtures of phenethylamines and caffeine, but not to their component drugs administered singly. Similarly, a cocaine-like stimulus could be produced by co-administering either the antihistamines doxylamine and diphenhydramine or combinations of dextromethorphan and diphenhydramine [11, 12]. Notably, David Gauvin and colleagues carried out many studies on compound drug-produced cues, incorporating training with drug mixtures from Gauvin et al. [13] onwards.

This review focuses upon the use of drug mixtures to establish stimulus control and does not attempt to comprehensively cover studies examining generalization in the opposite direction, when single drugs are for training and diverse mixtures are tested. Despite the greater complexity of studies with drug mixtures, it became clear at an early stage that the approach can yield orderly and interpretable data. Some of the questions to which answers were sought are listed here. Are the stimuli produced by mixtures of drugs perceived and processed in terms of the component substances or as new homogenous entities? Can the characteristics of cues produced by mixtures of drugs be manipulated by training according to different functional models for the relationship between drug stimuli and response? What are the effects of altering the doses and

relative amounts of the drugs used for training? Does the use of mixtures weaken the typically high specificity of drug-produced stimuli? What can be learned from studies with antagonists that selectively block the individual drugs in discriminable mixtures? What is the relevance of associative processes such as overshadowing and blocking that play key roles in the discrimination of compound exteroceptive stimuli? Do findings from research on drug mixtures have relevance to single drugs that have multiple effects?

B. FUNCTIONAL MODELS FOR THE DISCRIMINATIVE EFFECTS OF DRUG MIXTURES

Prior to considering evidence relating to the issues raised above, a number of different models are presented, building on the work of Järbe and Swedberg [14] and Stolerman [15]. These models focus attention on the functional relationships linking drugs to behavior and thus on procedural features that play a key role in determining the outcome of studies. Some examples of these models as they have been applied to studies of drug mixture discriminations are discussed here.

Figure 10-1A shows the basic *drug versus vehicle* model for studies in which the discrimination is between the dose of a single drug and the absence of that drug. The circled area represents the discriminative stimulus complex produced by the training drug, as distinct from the remainder of the perceptual space that is associated with administration of vehicle. The effects of the drug become associated with reinforcement of one of two mutually incompatible responses (R_1) whereas the absence of the drug becomes associated with the other response (R_2). The drug response (R_1) is elicited by, and only by, agents that resemble the training drug with respect to some significant pharmacological property; in contrast the vehicle response does not become selectively associated with R_2 because heterogeneous agents other than the training drug elicit R_2.

Drug mixture discriminations involve models in which a particular response is associated with more than one drug. Some studies have entailed reinforcing R_1 when a mixture (combination) of two dissimilar drugs has been administered (drugs A and B) and reinforcing R_2 after administering vehicle (Figure 10-1B). Stolerman [15] has called discriminations of this type AND-discriminations. The major characteristics of these discriminations have included full or almost full generalization to either drug A or drug B when they are administered separately (section D below). Discriminations may also be developed where either drug A or drug B (given separately on different occasions) is associated with R_1 and where vehicle is associated with R_2 (OR-discrimination, Figure 10-1C). In such cases full generalization is expected with agents from the different pharmacological classes of drugs A and B; OR-discriminations [16] allow the selectivity of antagonists to be tested with a single group of subjects and are mentioned here for logical completeness and to contrast with the discriminations involving drug mixtures.

There are also procedures that combine the features of the AND- and the OR-discrimination paradigms. Thus, Figure 10-1D shows the situation in which subjects are trained in such a way that R_1 is associated with administration of drug A and B as

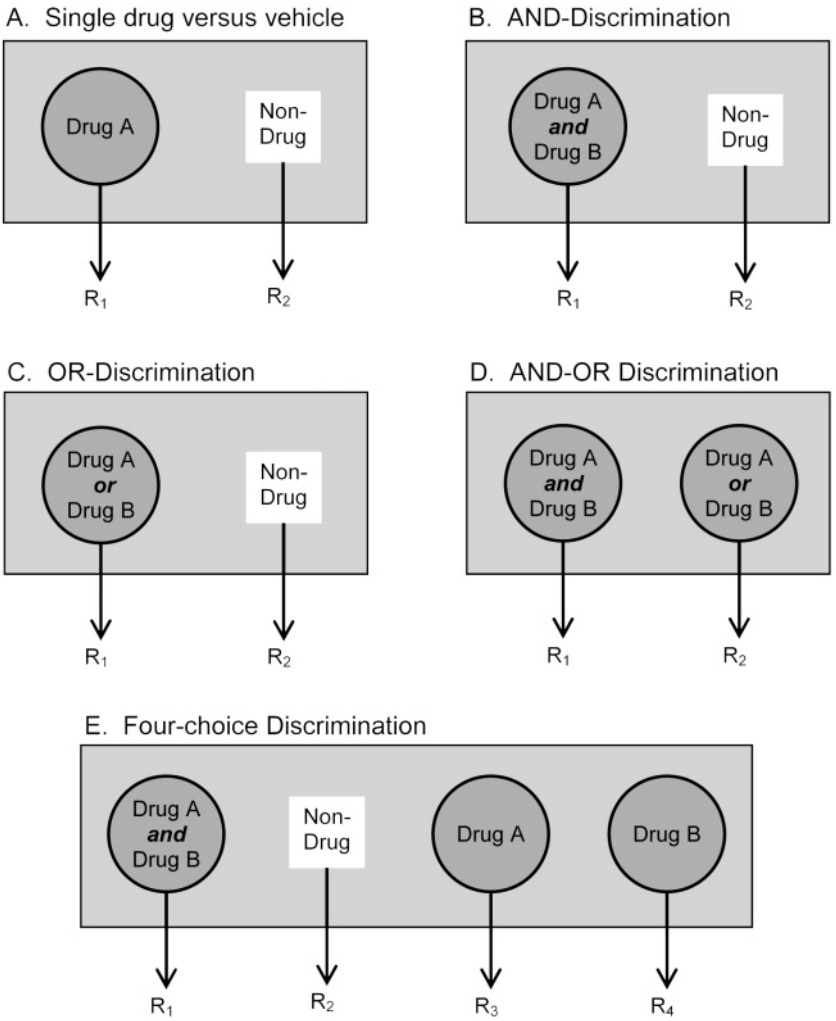

Figure 10-1. Schematic diagrams for five different drug discrimination paradigms produced by extending the concepts of Järbe and Swedberg [14] and Stolerman [15]. Upper section shows models that have been used in substantial numbers of studies on the discrimination of single drugs and mixtures of two drugs. Here R_1 represents the response associated with the training drug or drug mixture and R_2 represents the response associated with the non-drug state. Center section shows models for more rarely used discriminations involving two drugs. Lower section shows the model for a very rarely used four-choice discrimination procedure that includes a response associated with each of the four drug states used during training. "Drug A and Drug B" refers to the state produced by co-administration of two substances whereas "Drug A or Drug B" refers to the states produced by administering the two drugs on different occasions.

a mixture and R_2 is associated with the same drugs administered singly. This AND-OR discrimination procedure shows distinctive characteristics that have been exploited in a small number of studies (section F). Notably, subjects distinguish fully between the mixture and any dose of its component drugs. The AND-OR discrimination procedure allows identification of drugs that generalize only with the training mixture and not with the component agents given separately. However, the model suffers from the disadvantage that there is no response that is associated with the administration of vehicle; clearly, a significant issue for pharmacological studies.

All of the models considered above are for two-choice procedures that utilize only two manipulanda. Three and four choice procedures are also possible. A three-choice procedure might entail one response associated with vehicle, a second response associated with drug A or drug B given singly, and a third response associated with a mixture of the two drugs. Such three-choice procedures do not appear to have been used, but there are a small number of studies with the four-choice procedure shown in Figure 10-1E . This procedure has the considerable theoretical merit of distinguishing between novel compounds that (i) uniquely resemble the drug mixture, or (ii) that resemble either of the training drugs given singly, and (iii) other compounds from different pharmacological classes [17, 18]. The disadvantage is the relatively long time taken to train such discriminations as compared with two-choice procedures, whereas the training of drug mixtures with the two-choice AND-discrimination procedures takes no longer than the training of simple drug versus vehicle discriminations.

The range of possible procedures for studying drug mixture discriminations is wider than those described above. As noted by Stolerman [15], the full utilization of the approach's potential requires experimenters to devise novel paradigms where the innovation is not just pharmacological (e.g., the use of novel drugs) but extends to varying the behavioral models and thus the functional relationships between stimuli, responses and reinforcers. For example, Järbe et al. [19] conducted studies where one component in a compound stimulus was a drug and the other was an exteroceptive (visual) stimulus. At this point it is apposite to note that the terminology used above (AND-discrimination, etc.) is not the only one possible; Gauvin and Holloway [20] introduced the term plus-discrimination for the AND-discrimination paradigm. There is a case for further developing Gauvin's terminology, which is based on the logical relations between stimuli, whereas the approach of Stolerman [15] has the more limited benefit of providing easily remembered names for identifying different procedures.

C. INITIAL STUDIES: MIXTURES OF NICOTINE PLUS MIDAZOLAM

Studies considered in this section and in section D below used the AND-discrimination procedure. There was no reason to suspect any pharmacological interactions between nicotine and midazolam, and the stimuli produced by either drug alone had been investigated and, therefore, these substances were chosen for investigations on the discrimination of drug mixtures. Stolerman et al. [3] trained rats to discriminate (–)-nicotine plus midazolam from vehicle to 80% accuracy in a two-lever operant conditioning procedure. After the discrimination was acquired, both nicotine and midazolam produced

partial generalization when given separately. At the training doses, each drug alone produced 50–60% mixture-appropriate responding, as compared with 85% for the mixture. A larger dose of midazolam produced a score of 73%, whereas increasing the dose of nicotine suppressed responding altogether. The nicotine antagonist mecamylamine and the benzodiazepine antagonist Ro 15-1788 (flumazenil) only slightly (20–30%) attenuated the discriminative response to the mixture when given separately, but completely blocked the response when co-administered [3]. In rats trained to discriminate the same doses of nicotine or midazolam from vehicle in conventional single-drug discriminations, nicotine did not affect the dose-response curve for midazolam and *vice versa*, supporting the assumed lack of pharmacological interactions when effects of mixtures were assessed. While each component of the mixture cue may itself have been comprised of a discriminative stimulus complex, it seemed logically sound to treat each of these complexes as a unit for the purposes of the study. Thus, it appeared that the two elements of the training mixture were perceived separately, rather than being blended into a homogeneous novel entity. These findings supported those of Hanlin and Appel [9] for an amphetamine-LSD mixture and were consistent with studies on compound exteroceptive stimuli [21]; using Mackintosh's terminology, this might be called a redundant discrimination in the sense that the drug stimulus could be identified on the basis of either one of its components. However, generalization to the component drugs at the training doses was partial, so to some extent resembled a complex discrimination in the sense that the term was used by Mackintosh [21].

The partial generalizations to the training doses of nicotine and midazolam were confirmed by Garcha and Stolerman [22] in a study that also demonstrated the absence of generalization to amphetamine, morphine, or quipazine, up to doses that reduced overall rates of responding. A discrimination based on a drug mixture was therefore shown to retain at least some of the pharmacological specificity seen in discriminations based upon its component drugs. Other reports using diverse drugs from a range of pharmacological classes for training AND-discriminations have typically found that selectivity is retained [e.g., 23, 24, 25, 26].

Differences between specificity of the AND- and the AND-OR procedures are discussed in sections F.2 and F.3. Knowledge of the characteristics of compound drug-produced stimuli may aid interpretation of the discriminative effects of single drugs with broad spectra of action, an aspect discussed in section I below in relation to ethanol.

D. CHARACTERISTICS OF DIVERSE DRUG MIXTURE DISCRIMINATIONS

1. Psychomotor Stimulants plus Depressants

The first set of studies discussed here were based on discriminations of amphetamine plus pentobarbital. Witkin et al. [6] trained one group of pigeons to discriminate a mixture of amphetamine plus pentobarbital from amphetamine alone whereas other birds were trained to discriminate the mixture from pentobarbital. In earlier behavioral

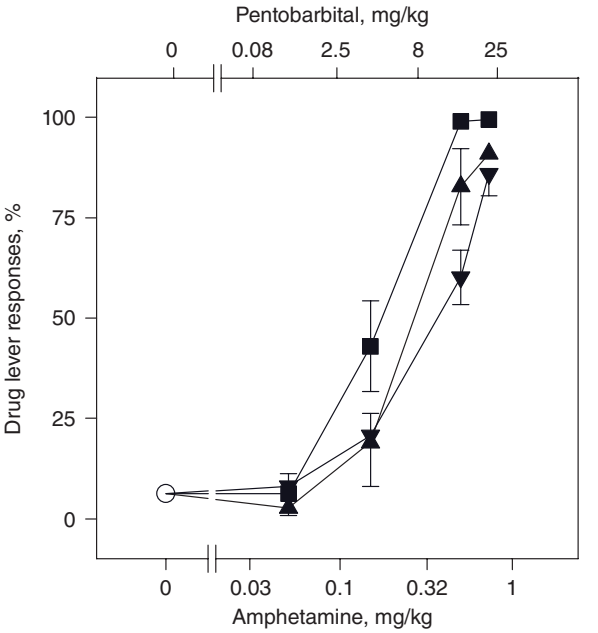

Figure 10-2. Dose-response curves for rats trained to discriminate a mixture of 0.5 mg/kg of (+)-amphetamine plus 12 mg/kg of pentobarbital from saline (n = 9). Results are shown for the mixture of the two drugs (■), for (+)-amphetamine alone (▲), for pentobarbital (▼) and for saline (○). Lower abscissa shows doses of amphetamine, upper abscissa shows doses of pentobarbital. Ordinate shows responses on the drug-appropriate lever as a percentage of total responses on both levers. Data were obtained in 5-minute extinction tests. All injections were SC, results are means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted (redrawn from data of [23]).

experiments, amphetamines and barbiturates were found to interact in additive, synergistic, or antagonistic ways depending upon behavioral baselines and other factors [27, 28]. It was hypothesized that such mixtures might exhibit unique discriminative stimulus properties that could explain why they were particularly subject to abuse.

A discriminative stimulus based of a mixture of (+)-amphetamine plus pentobarbital showed partial generalization when either component drug was given at the dose in the training mixture [23]. At larger doses there was complete generalization to each component alone (Figure 10.2), but there was little cross-generalization between the amphetamine plus pentobarbital in rats trained with each drug separately. Study not shown. The studies in rats trained to discriminate amphetamine or pentobarbital alone did not yield any substantial evidence for pharmacological interactions between the two drugs. These findings were extended and modified in a later study [29]. To estimate the effects of discriminated mixtures from those of their component drugs, responses to the components were combined according to standard rules for combinations of probabilities [21, page 575]. The predicted response probability was calculated as

$R_M = (R_A + R_B) - (R_A \times R_B)$, where the subscripts denote responses to the mixture (M) and its components (A and B). Predicted responses closely matched actual responses across a three-point dose-response curve [23]. Such findings suggested that components of the compound stimulus were processed separately rather than being blended into a new and distinct stimulus. Rules governing discrimination of the mixture of amphetamine plus pentobarbital appeared generally similar to those found in previous studies with nicotine and midazolam, drugs that were not known to be subject to abuse as a mixture. As was the case for mixtures of nicotine plus midazolam discussed above, generalization to the training drugs was partial at the doses used for training and tended towards completeness at larger doses. However, more extensive dose-response studies discussed below (section E) did reveal potentiation at different dose combinations of amphetamine plus pentobarbital.

Other mixtures of psychomotor stimulant and depressant drugs have also been examined. Hutchinson and Riley [30] reported, in abstract form only, that rats trained to discriminate a mixture of cocaine and ethanol from vehicle generalized fully to cocaine and partially to ethanol. Rats trained to discriminate a mixture of nicotine plus ethanol showed complete (88%) generalization to nicotine and slightly weaker (75%) generalization to ethanol [20]. Interestingly, two pigeons trained to discriminate a mixture of morphine and amphetamine from vehicle did not generalize at all to amphetamine and only generalized reliably to doses of morphine that were larger than the dose used for training [31]. The results were suggestive of a supra-additive interaction between the drugs, a concept that called for more extensive testing to enable statistical validation.

2. Mild Stimulants

The discriminative effects of mixtures of caffeine and the mildly stimulatory appetite suppressant phenylpropanolamine (PPA) were investigated because these drugs had been abused together. Discriminations of mixture, caffeine alone, and PPA alone were 90% accurate after 40 sessions [32]. Generalization from the mixture to either PPA or caffeine was weak (25–47%) at the doses used in the training mixture, although there was almost complete generalization to larger doses of PPA. Responses to different amounts of the mixture were calculated from the responses to the corresponding doses of the component drugs according to the equation in section D.1 above. When tested at the training doses, the actual response to the mixture of 94% was significantly greater than the predicted response of 65%. This suggested a possible synergistic interaction between caffeine and PPA because the discriminative effect of the mixture could not be fully explained by the combined effects of its component drugs. However, in rats trained on caffeine, PPA had no effect on the dose-response relationship for caffeine; similarly, in rats trained on PPA, caffeine had no effect on the dose-response relationship for PPA. Therefore training with, or perhaps just repeated exposure to, the mixture seemed to influence its characteristics.

Generalization to (+)-amphetamine or cocaine was weakest in rats trained on caffeine, was partial in rats trained on the mixture, and was complete in rats trained on PPA; thus, the mixture of caffeine and PPA was not more similar to either cocaine or amphetamine than it was to PPA alone [32].

The results discussed above were in agreement with reports that caffeine and PPA may interact in a complex manner, but did not support the view that this enhanced their resemblance to typical psychomotor stimulants as was suggested previously [10, 33]. An extensive study by Gauvin et al. [13] also showed that the stimuli produced by mixtures of caffeine, PPA and ephedrine were based upon supra-additive interactions. This report included one of the rare instances where a mixture of three drugs was used for training a discrimination. Gauvin et al. [25] also reported on partial generalizations to the component drugs of mixtures containing dextromethorphan and either ephedrine or diphenhydramine. These training mixtures generalized fully to amphetamine or cocaine, suggesting the potential abuse liability of some common over-the-counter drug mixtures.

3. Phentermine plus Fenfluramine

Clinical observations suggest that the combined administration of fenfluramine and phentermine was useful for treating both alcohol and cocaine dependence. Shoaib et al. [24] trained rats to discriminate a mixture of these drugs from vehicle to test for possible interactions between them. Rats acquired the mixture discrimination rapidly, while in rats trained on each drug alone it seemed to be necessary to increase the training doses to obtain good stimulus control. The component drugs of the mixture generalized partially to the mixture at their respective training doses, and completely at larger doses. Interestingly, microdialysis showed that the degree to which each amine substituted for another in the cross-generalization tests was correlated with elevations of dopamine and 5-hydroxytryptamine (5-HT) overflow from the nucleus accumbens. There also seemed to be a simple addition of neurochemical effects with the mixture; each of the neurotransmitters was increased to the level observed when the compounds were given alone.

In rats trained to discriminate fenfluramine alone, doses of phentermine and of the mixture also increased drug appropriate responding, but only to a maximal level of 50%. The co-administration of phentermine seemed to weaken the generalization that would have been expected due to the fenfluramine in the mixture; pharmacological antagonism seemed unlikely and therefore the effect may have reflected perceptual masking of one stimulus by another [24]. In rats trained with phentermine alone, there was no generalization to fenfluramine, and tests with the mixture showed increases in drug-appropriate responding that were similar to those seen with phentermine alone. The mixture-trained rats showed full generalization to cocaine and partial generalization to amphetamine, largely reflecting the results obtained in rats trained on fenfluramine or phentermine alone. From the preceding results Shoaib et al. [24] concluded that the two drugs given as a mixture did not produce a novel cue but appeared to interact additively. It was also suggested that the therapeutic efficacy of the mixture may have been due to the combined effects of dopamine and 5-HT release and the additive relationship may have been an important feature of the treatment.

4. Mixtures Containing Opioids

The abuse of cocaine and heroin together (“speedballs”) has been known for decades. Mixtures of these drugs have been evaluated in rhesus monkeys and rats trained to

discriminate mixtures with a 10 : 1 ratio of cocaine to heroin. Either cocaine alone or heroin alone substituted completely for the cocaine/heroin mixture in both species [26, 34] but the demonstration of synergism in monkeys would have required additional data. However, an isobolographic analysis of data from the rats showed that a 1 : 1 mixture of heroin and cocaine produced supra-additive effects, whereas mixtures containing lesser proportions of heroin generated only additive effects (Negus, personal communication). Studies in animals trained to discriminate cocaine alone yielded little or no evidence for enhancement of the discriminative stimulus effects of cocaine by some m-opioid agonists, or vice versa. However, not all compounds that generalized with cocaine or m-opioid agonists in single-drug discrimination experiments substituted for the mixture of cocaine and heroin. These findings suggested that the stimulus effects of the mixture of cocaine and heroin did not overlap completely with those of the separate drugs [26].

Stolerman et al. [35] reported that rats trained to discriminate a mixture of morphine and midazolam partially generalized to the training doses of either drug alone, and a similar result was obtained with a mixture of pentazocine and tripelennamine (abused as "Ts and Blues"). In contrast, Carlezon et al. [36, 37] found that after training a discrimination of morphine and dizocilpine (MK-801) there was no generalization to either morphine or dizocilpine at the training dose, although dizocilpine, above the training dose, showed partial generalization. It was proposed that the combination of morphine and dizocilpine acted like a third drug that had unique discriminative stimulus effects, while retaining the analgesic and locomotor-stimulating effects of its components [37]. Shoaib and Stolerman [38] confirmed these findings and found that the responses to the training mixture were greater than those expected by combining the responses to the separate drugs; the interaction was greater than for any pair of drugs tested previously in the procedure. The pharmacological requirements for this effect are unknown; merely the presence of either morphine or dizocilpine in the training mixture is insufficient to produce such an interaction because rats trained to discriminate other mixtures containing just one of these drugs were able to identify the component stimuli [35, 39].

A small number of studies with opioids have used a four-choice procedure as represented diagrammatically in Figure 10-1E. For example, pigeons trained to discriminate between vehicle, morphine, pentobarbital and a mixture of the two drugs performed the task with high (90%) accuracy [40]. Small doses of all drugs given alone produced vehicle-appropriate responding, as did methamphetamine. Larger doses of pentobarbital or chlordiazepoxide produced responding on the pentobarbital key, whereas larger doses of morphine produced morphine-appropriate responding. A mixture of chlordiazepoxide with morphine produced mixture-appropriate responding. Mixtures of methamphetamine with pentobarbital or with morphine engendered responding similar to that produced by pentobarbital or morphine given alone. Additional studies using opioids and four-choice procedures are discussed in section F.1 below.

5. Mixtures Containing Sedative-Hypnotics and Other Depressants

Very few studies have appeared in this area. There would seem to be substantial reason for investigating many different mixtures containing, for example, ethanol and

inhalants, as well as clinically used substances such as anxiolytics, sedative-hypnotics, and antipsychotics. Metcalf et al. [41] examined the discrimination of a mixture of γ-hydroxybutyrate and ethanol, drugs that were subject to abuse together. The rats exhibited partial generalization to each component drug of the mixture when they were administered at the training doses and full generalization at larger doses. There was a lack of cross-generalization between γ-hydroxybutyrate and ethanol (which was surprising in view of previous reports) but there was some evidence of a synergistic interaction when they were administered as a mixture to rats trained on either drug alone. The complex nature of the stimulus properties of these drugs merits further investigation.

E. ROLE OF TRAINING DOSES

The dose of a single drug used to train a discrimination can influence quantitative aspects of the resulting cue (as assessed by ED_{50} values) and the qualitative nature of the cue, as shown by the extent of generalization to drugs from different pharmacological classes (see Chapter 3). The situation is more complex with cues based upon mixtures of dissimilar drugs, where the ratio between the doses of the two substances has to be considered as well as the absolute amount given of a mixture when the dose ratio is held constant. Few studies have addressed these questions and knowledge is therefore extremely patchy. These studies are summarized here.

Garcha and Stolerman [22] trained rats to discriminate a mixture of (–)-nicotine and midazolam. Tests with each drug administered separately showed that stimulus control was mainly (65%) attributable to the effects of midazolam rather than to the actions of nicotine (25%). The animals were then retrained with a large dose of nicotine and a sequence of decreasing doses of midazolam. As the training dose of nicotine increased and that of midazolam decreased, the magnitudes of responses to the training doses of the separate drugs were progressively reversed, until stimulus control by the mixture was mainly attributable to the effects of nicotine (85%) and the contribution of midazolam effects became very minor (15%). The initial results were reproduced upon reversion to the original training doses. A similar study was carried out with (+)-amphetamine plus pentobarbital but in this case the training dose of amphetamine was held constant throughout while that of pentobarbital was increased sequentially [23]. Initially, stimulus control was attributable almost entirely to the effects of amphetamine but the action of pentobarbital took over control as its dose was raised, and at the same time the response to amphetamine was attenuated (Figure 10-3).

Thus, responses to the components of the compound stimuli produced by two different drug mixtures were systematically related to the amounts of drugs in the mixtures used to maintain the discrimination. In both cases only a fairly small, 4:1 change in the dose ratio was necessary to completely reverse the relative importance of the components of the stimulus effects. There was also some evidence that a strong stimulus produced by one drug may have overshadowed a weaker stimulus produced by a different agent; at certain dose ratios, nicotine or amphetamine exerted very little stimulus control despite the fact that the doses used could maintain good stimulus control when

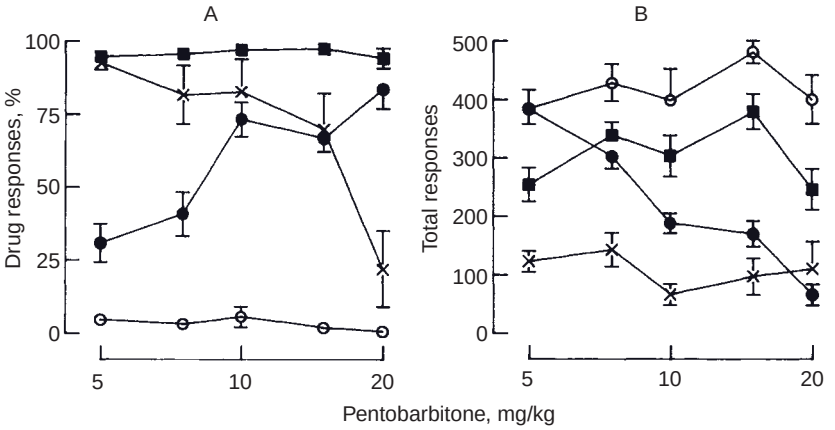

Figure 10-3. Discriminative stimulus effects of (+)-amphetamine (×) and pentobarbital (●) in rats trained to discriminate mixtures of these drugs (■) from saline (○). Results are shown for five sets of tests carried out while stimulus control was maintained by mixtures containing different doses of pentobarbital (n = 12). Dose of (+)-amphetamine was 0.4 mg/kg throughout. Abscissae, doses of pentobarbital. (A) responses on the drug-appropriate lever as a percentage of total responses on both levers; (B) total responses on both levers. Other details as for Figure 10-2 (reproduced from [23]).

used as the sole training drug. The possibility that this was due to overshadowing rather then pharmacological antagonism was investigated more extensively (section H.1).

A later study investigated the impact of varying the dose of a mixture with a constant ratio between the doses of its component drugs [29]. Three groups of rats were trained to discriminate mixtures of amphetamine plus pentobarbital from vehicle to 90% accuracy (dose ratio = 1 : 25 throughout). There was almost full generalization to the training doses of amphetamine alone in rats trained with mixtures of the two smaller doses of the single drugs, but response rate suppression limited the data available from the third group. Generalization to pentobarbital alone was 39% in the rats trained with the smallest absolute dose of the mixture and greater (75–77%) after training at the two larger doses of the mixture, despite the fact that the ratio between the doses of the drugs was held constant. This finding reflected the relatively steep dose-response curve for pentobarbital in conventional, single-drug, discriminations.

Doses of pentobarbital that were half of those used for training produced little discriminative response when administered alone to rats trained with the two smallest doses of the mixture; the same doses of pentobarbital strikingly increased responses to amphetamine in a supra-additive manner. This difference from the findings of Mariathasan et al. [23] may be attributable to the different dose ratios examined in the two studies (see section F.1. below). The specificity of the discriminations was explored further. There was partial generalization when either apomorphine (50%) or nicotine (63%) was administered alone, and these responses were largest in rats trained with the

smallest dose of the mixture [29]. Strikingly, some doses of apomorphine and pentobarbital that did not generalize at all when administered separately produced full generalization when administered together, but only in rats trained with the smaller doses of the mixture. Pentobarbital did not enhance generalization to nicotine in any group. Thus, patterns of generalization to single drugs followed an orderly pattern resembling those for discriminations established with single drugs. However, no simple rules to predict the influence of training dose on generalization from one mixture to another were ascertained.

F. VARIATIONS IN FUNCTIONAL RELATIONSHIPS: THE ROLE OF TRAINING PARADIGM

1. Basic Characteristics of AND-OR and OR Discriminations in Rats

The experiments discussed in sections D and E above involved training AND-discriminations of drug mixtures versus vehicle. The studies reviewed in this section investigated the impact of different and rarely studied functional relationships between the drugs, responses and reinforcers. Stolerman and Mariathasan [43] reported success in training rats under an AND-OR discrimination paradigm (Figure 10-1 D). In these animals either (+)-amphetamine or pentobarbital given separately engendered responding upon one lever whereas a mixture of the two substances supported responding upon a different lever. Figure 10-4A shows that in contrast to the AND-discrimination with the same substances, no dose of either drug alone engendered any responding upon the mixture-appropriate lever. Furthermore, these characteristics persisted for a prolonged period after the animals were switched to training under AND-discrimination conditions (Figure 10-4B).

Mariathasan and Stolerman [42] extended these observations by directly comparing the performance of rats discriminating amphetamine and pentobarbital in the AND-, the OR- and the AND-OR discrimination procedures (Figure 10-1, B–D). Rats trained under the AND procedure acquired the discrimination more rapidly than the animals trained on the other two procedures but after 60 training sessions, all discriminations were performed with similar (90–94%) accuracy. In accordance with previous work with the AND-discrimination procedure, there was full generalization from the mixture to the largest doses used of either amphetamine or pentobarbital. In contrast, under the AND-OR procedure, there was no generalization from the mixture to any dose of either drug given separately, confirming the findings of Stolerman and Mariathasan [43]. In the OR-discrimination procedure, responding on one lever was reinforced after administration of amphetamine or pentobarbital separately or as a mixture, whereas responding on the other lever was reinforced after vehicle. In accordance with these contingencies, either amphetamine alone or pentobarbital alone produced dose-related increases in responding on the drug lever. The data demonstrated a very powerful influence of the training paradigm on the characteristics of a drug mixture discrimination. While the differences between paradigms were very clear, they were, in a sense, unsurprising; in each case, subjects performed strictly according to requirements of the contingencies of reinforcement that were in place during training.

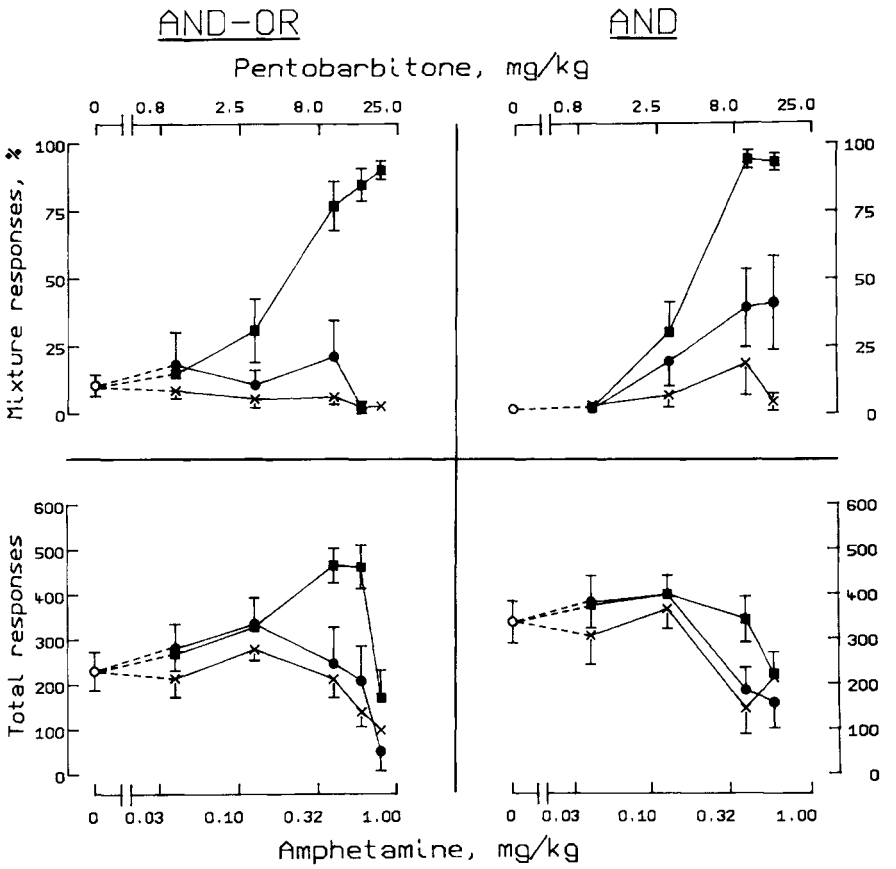

Figure 10-4. Dose-response curves for eight rats trained to discriminate a mixture of 0.4 mg/kg of (+)-amphetamine and 10 mg/kg of pentobarbital from either drug separately (left section, AND-OR discrimination) or at a later stage in their history, from saline (right section, AND-discrimination). Upper section shows discriminative stimulus effects as percentage responding on the mixture-appropriate lever; lower section shows total responses on both levers. Results are shown for amphetamine (×) and pentobarbital (●) given separately and as a mixture (■) and for saline (○). Data were obtained in 5-minute extinction tests. All injections were SC, results are means ± SEM, with overlapping SEM and those smaller than diameters of symbols omitted (reproduced from [43]).

Interestingly, in the presence of a small dose of pentobarbital to which there was no generalization, doses of amphetamine that were below threshold for generalization enhanced mixture-appropriate responding in rats trained in the AND and OR procedures. This potentiating effect of amphetamine under AND-discrimination conditions was not seen by Mariathasan et al. [23] who did not test mixtures with different ratios between the doses of component drugs, but was detected in both of the studies that

varied the ratios between doses of the drugs in mixtures tested for generalization [29, 42]. Interestingly, Dickins et al. [44] found a higher frequency of reports of euphoria with certain mixtures of amphetamine plus amylobarbital than with the separate drugs; these effects, like the enhanced motor activity of rats after co-administration of amphetamine and amylobarbital [27], were also highly dose-dependent.

The different training conditions also produced changes in rates of responding. Rats trained under the AND-OR procedure responded at a relatively low rate after administration of vehicle [42, 43]. This effect was thought to resemble low response rates seen in tests with vehicle in drug versus drug discrimination procedures that do not entail training with mixtures of substances (e.g., [45]). The effect may be attributable to the discriminable difference between the non-drug condition and all other stimuli presented during training; there is no correct response for the non-drug state and animals may, therefore, have a tendency to withhold responding.

Procedures offering three or four response choices (e.g., Figure 10-1E) may be seen as elaborations of the two-choice AND-OR procedure. They avoid effects due to the absence of a vehicle-appropriate response but require extended periods of training even when the subjects are pigeons (that often acquire complex tasks quickly). Using a four-choice procedure, Li et al. [18] trained pigeons to discriminate between pentobarbital, amphetamine, a mixture of these two drugs, and vehicle. After receiving small doses of either pseudoephedrine or nicotine, the birds responded on the saline key. After larger doses of these drugs, responding occurred primarily on the amphetamine key; this observation reflected the known similarities between the stimulus effects of the drugs that could be detected in single-drug discrimination procedures. Interestingly, in pigeons trained to discriminate between vehicle, morphine, methamphetamine, and a mixture of these two drugs, the larger doses tested of pseudoephedrine and especially of nicotine engendered rather more mixture-appropriate responding. Li et al. [18] concluded that "The four-choice procedure can reveal subtle effects in the discrimination of individual drugs and drug combinations that are not apparent with procedures offering fewer response alternatives." It was also found that pigeons responded on the mixture key even when dosed with mixtures below the doses of the drugs used in the training mixture, a finding that was not readily predictable from the results of two-choice procedures.

Pigeons were also trained to discriminate between vehicle, morphine, the κ-opioid agonist U-50,448, and a mixture of these drugs [40]. Mixtures of small doses of both drugs produced responding on the vehicle key, but mixtures containing larger doses of U-50,488 produced responding on the U-50,448 key. Similarly, mixtures of the larger doses of morphine with small doses of U-50,448 produced responding on the morphine key, whereas mixtures containing larger doses of U-50,448 produced progressively more responding on the mixture key. The four-choice procedure is a novel way to study mixed agonists that act at both mu and kappa opioid receptors.

2. Specificity of Drug Mixture Discriminations: Single Drug Substitution Tests

After stimulus control is acquired, generalization tests may be carried out with either single drugs or mixtures. In this section the impact of training paradigm on the results

of generalization tests with single drugs is considered first, followed by tests with mixtures where only one novel component drug is introduced. The specificity of drug mixture discriminations has been compared in studies utilizing the AND and the AND-OR procedures. After stimulus control with amphetamine plus pentobarbital was acquired under AND discrimination procedures, either midazolam or nicotine administered singly partially generalized, whereas no dose tested of caffeine, cocaine, or ethanol had such an effect. The extent of these generalizations can also depend upon the doses of drugs used for training as noted in section E. The preceding findings from tests with single drugs in the AND-discrimination procedure can in many instances be understood by referring to previous work on discriminations supported by the individual drugs in the current training mixtures. For example, it is known that rats trained to discriminate amphetamine but not barbiturates show partial or even full generalization to nicotine [46, 47]; thus partial generalization to nicotine in rats trained on an amphetamine-barbiturate mixture is probably associated with the stimulus effects of amphetamine. Conversely, the partial generalization to midazolam in such rats is probably associated with the effects of pentobarbital in the training mixture since barbiturates can generalize with midazolam in rats, but amphetamine does not [48, 29]. Other studies also suggest that when a test drug has discriminative effects that resemble those of one training drug, partial or full generalization may occur [13, 32, 103]. Thus, the failure to see generalization to caffeine from an amphetamine-barbiturate training mixture is not surprising since neither component drug generalizes with it [50, 51]. The lack of generalization to ethanol alone may reflect observations of incomplete cross-generalization between barbiturates and ethanol [52, 53]. The failure to see any generalization to cocaine is not easily explained since there are many reports of full generalization between amphetamine and cocaine (e.g., [54, 55]), although it has been claimed that there are minor differences between their stimuli [55, 56].

In rats trained with a mixture of amphetamine plus pentobarbital in the AND-OR discrimination procedure, none of the novel drugs given singly (nicotine, cocaine, caffeine, midazolam and ethanol) produced any increase in mixture-appropriate responding [57]. This was consistent with the complete dissociation of the effects of the training mixture from those of its component drugs in this procedure [29, 42, 43]. If even the component drugs do not produce mixture-appropriate responding at any dose, then it is not surprising that other single substances whose discriminative effects are, to varying extents different from those of the component drugs also do not engender mixture-appropriate responding. The absence of generalization to any of the single drugs tested under these conditions was therefore predictable and interpretable.

Results of generalization tests with mixtures containing one of the training drugs and one novel component become more complex [57]. In the AND discrimination procedure, there was full generalization from training mixtures of amphetamine plus pentobarbital to mixtures of cocaine or nicotine with pentobarbital. Similarly there was full generalization to amphetamine plus midazolam. When the same mixtures were tested in rats trained under the AND-OR procedure, there was full generalization only to cocaine plus pentobarbital, with partial generalization to nicotine plus pentobarbital or to amphetamine plus midazolam. These observations suggested that training under

the AND-OR procedure produced a discrimination displaying greater pharmacological specificity than training under the AND procedure.

3. Specificity of Drug Mixture Discriminations: Dual Drug Substitution Tests

Very few studies have examined generalizations from one mixture of drugs to novel mixtures in which either or both of the components have been changed ("dual substitution" tests). A ground-breaking study by Gauvin and Holloway [20] was a notable exception; rats trained to discriminate a mixture of nicotine plus ethanol from vehicle in an AND-discrimination procedure generalized fully to a mixture containing two different drugs, amphetamine plus pentobarbital. This result may relate to reports of cross-generalization between amphetamine and nicotine [58] on the one hand, and between ethanol and pentobarbital [53] on the other; it may have implications for hypotheses that tobacco and alcoholic beverages act as gateways to the abuse of illicit drugs.

Later investigations directly compared the results of dual substitution test results under AND and AND-OR procedures [57, 59]. In rats trained to discriminate a mixture of amphetamine plus pentobarbital, mixtures of either nicotine plus midazolam or caffeine plus ethanol produced very marked generalization under AND-discrimination conditions. However, the same mixtures did not increase mixture-appropriate responding in rats trained under the AND-OR procedure. In rats trained to discriminate a mixture of nicotine plus midazolam, a mixture of amphetamine plus pentobarbital generalized fully (90%) under AND-discrimination conditions but only partially (51%) in rats trained under the AND-OR procedure (Figure 10-5).

4. Impact of Prior Training in the AND-OR Discrimination Procedure

In the final phase of the study by Mariathasan and Stolerman [42], all rats were retrained to discriminate the mixture from vehicle under the AND-discrimination procedure. There was no difficulty in obtaining reliable discriminative performance regardless of previous training. The accuracy of lever selection for the first 10 training sessions at this stage of the study was 95, 96, and 96%, for the former AND, or and AND-OR groups, respectively. Dose-response curves for amphetamine plus pentobarbital were obtained after training the rats under the altered procedures for 30 sessions. In all three groups, there was very little mixture-appropriate responding after administration of vehicle, whereas the response to the mixture at the dose used for training was 92–98%. Either amphetamine or pentobarbital given separately increased mixture-appropriate responses in the former AND and OR groups. However, in the former AND-OR group, the separate drugs did not increase mixture-appropriate responding at any dose tested, confirming the findings of Stolerman and Mariathasan [43] and showing that previous behavioral history influenced the characteristics of the cue obtained.

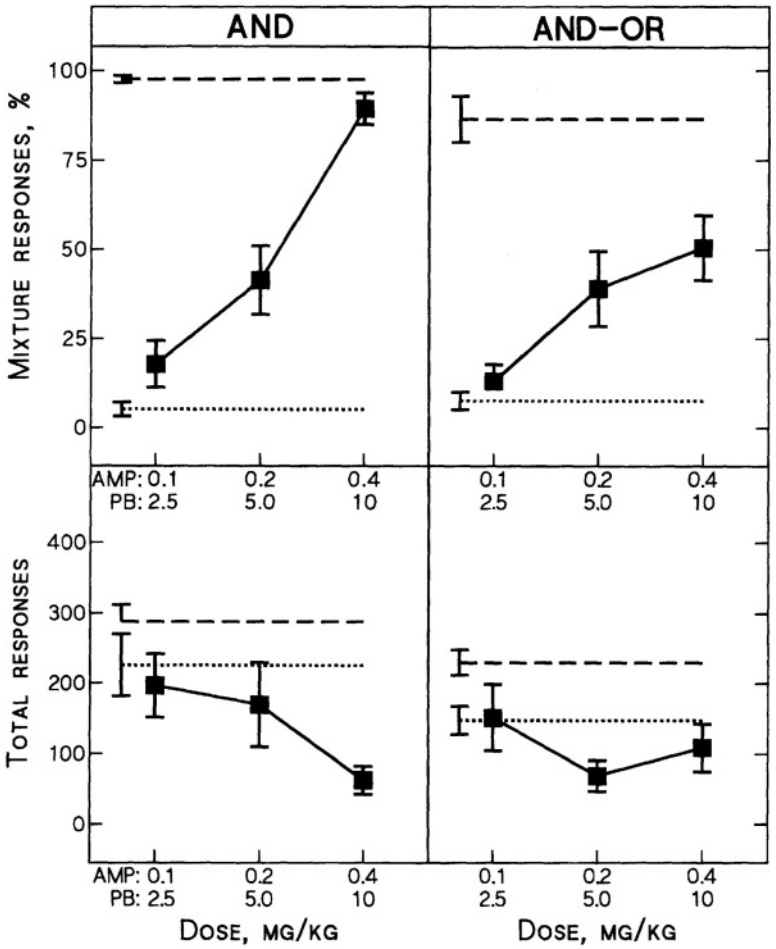

Figure 10-5. "Dual-substitution" generalization tests in rats trained with a mixture of nicotine (0.4 mg/kg) plus midazolam (0.2 mg/kg) under AND and AND-OR discrimination procedures (n = 10). Results are shown for mixtures containing three doses of amphetamine (AMP) plus three doses of pentobarbital (PB) as indicated by the dual scales on the abscissae (■). Horizontal lines represent responses to saline (….) and training mixture (—-). Other details as for Figure 10-4 (reproduced from [59]).

5. Summary

Several lines of evidence presented above suggest that AND-OR training increases the specificity of drug discriminations. First, with this procedure, there is no generalization from the mixture to any dose of its constituent drugs given separately. When novel drugs are tested singly, generalization is also absent. Next, when novel drugs are tested, generalization in both single substitution tests and dual substitution tests is substantially

attenuated. In every instance where comparisons were made, generalization was either greater, or occurred at lower doses and over a wider dose range under the AND than under the AND-OR discrimination procedure. Finally, the effectiveness and potency of specific receptor antagonists is increased in the AND-OR procedure (see section G below). Other methods that increase specificity have been described, including drug versus drug training [60] and three-lever discriminations [61, 62, 63]. Perhaps the closest to the AND-OR procedure is the approach of Overton [64] that entailed training one drug versus two to three other, different substances; however, in Overton's experiments, drug mixtures were not used as training stimuli.

The enhanced specificity of the AND-OR mixture discrimination may be a significant advantage for comparisons of the stimulus properties of abused mixtures. When a novel abused mixture is tested for generalization in rats trained with a standard mixture (i.e., a dual substitution test), the AND-discrimination procedure is likely to result in full generalization if only one of the substituted novel drugs is identical in effect to either drug in the training mixture; such a result might reasonably be considered as a "false positive" in pharmacological terms. Even one identical and one inert drug can produce such a result. In contrast, with the AND-OR discrimination, the effects of both drugs must be reproduced for full generalization to occur. It may be the case that full generalization in the AND-OR procedure can be obtained only if the substitute drugs reproduce not only the effects of each individual training drug, but also any novel stimulus condition that might be associated with a nonadditive interaction between them.

G. ANTAGONISM OF MIXTURE CUES AND TRAINING WITH AGONISTS PLUS ANTAGONISTS

The pharmacological validity of drug mixture discriminations would be compromised if specific antagonists did not produce an orderly pattern of results. In studies of nicotine plus midazolam discrimination using the AND procedure [3, 65], neither a benzodiazepine antagonist alone nor a nicotinic antagonist alone produced more than a marginal and unconvincing block of the discriminative effects of a mixture; nevertheless, both components were clearly important elements in the stimulus complex. When the two antagonists were co-administered there was a complete block of the response to the mixture. Comparable results were obtained using the antagonists methysergide and haloperidol in rats trained to discriminate a mixture of fenfluramine and phentermine [66]. In rhesus monkeys discriminating a mixture of heroin plus cocaine, co-administration of flupenthixol plus quadazocine was much more effective than either antagonist alone [26]; doses of these antagonists that had no effects on their own produced clear rightward shifts in dose-response curves for the speedball mixture, and responding was shifted entirely to the vehicle-appropriate key at some combinations of the mixtures.

It may be inferred that in drug discrimination generally, weak degrees of antagonism may reflect real effects deserving further investigation. Consider also the study of Appel et al. [67], who found that the discriminative effects of the opioid pentazocine

could be fully blocked only by a mixture of a dopamine antagonist and a narcotic antagonist. This is precisely what would be predicted by extending the results for antagonism of drug mixtures to antagonism of the single drug pentazocine that has multiple effects. Similarly, dopamine antagonists produced only a weak blockade of the nicotine discriminative stimulus complex, in which dopamine release was thought to be just one component. However, findings with nicotinic α7 receptor knockout mice supported the concept of an α7 nicotinic receptor-mediated dopaminergic element in nicotine discrimination [68]. These animals were able to discriminate nicotine like wild-type controls but partial generalization to amphetamine, an indirect dopamine agonist, was attenuated.

The antagonism of a mixture of nicotine plus midazolam has also been compared under the AND and the AND-OR procedures (Figure 10-6). The antagonist effects of both mecamylamine and flumazenil given alone were more marked in rats trained under

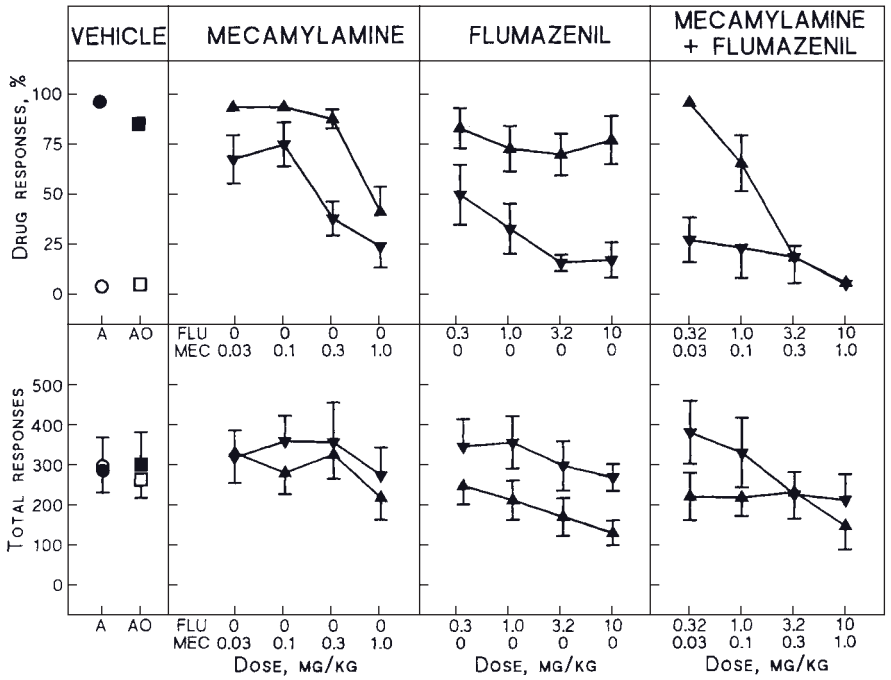

Figure 10-6. Impact of mecamylamine (MEC), flumazenil (FLU) and mixtures of mecamylamine plus flumazenil on discrimination of a mixture of nicotine (0.4 mg/kg) plus midazolam (0.2 mg/kg) under two training procedures. Results are shown for rats trained to discriminate mixture from saline (AND-discrimination, n = 8) and mixture from either component drug alone (AND-OR discrimination, n = 6). Points above A and AO in panels on the extreme left of figure show responses to saline (○, □) and the training mixture (●, ■) for rats trained under AND and AND-OR procedures respectively. Other details as for Figure 10-4 (reproduced from [101]).

the AND-OR procedure than in rats trained on the AND-discrimination. Mixtures of mecamylamine plus flumazenil were also much more potent under the AND-OR than under the AND-discrimination procedure. In fact, the AND-OR paradigm reduced the dose of the antagonist mixture needed to produce complete block by a factor of about 10, as compared with the AND-discrimination. These striking differences in sensitivity to antagonists support the view that AND-OR or related procedures may enhance the pharmacological specificity of complex drug discriminations.

Some studies have examined the impact of training with mixtures of receptor agonists and antagonists. Bemegride attenuated the acquisition of pentobarbital discrimination in a T-maze shock-escape procedure [69]. Other reports have described the block of morphine and physostigmine discriminations by naltrexone and Ditran, respectively [2, 5]. Ditran is itself a mixture of structural isomers and although they have broadly similar anticholinergic properties, it is clear that Ditran is not an ideal antagonist. A later study examined the effects of training with a wide range of doses of the noncompetitive nicotine receptor antagonist mecamylamine on the discrimination of (–)-nicotine using a two-lever operant conditioning procedure with food reinforcement [70]. Mecamylamine (0.1–0.8 mg/kg) impaired accuracy during the acquisition of the nicotine discrimination in a dose-related manner. In generalization tests, rats trained with nicotine alone yielded a typical nicotine dose-response curve (ED_{50} = 0.082 mg/kg). In rats trained with nicotine plus 0.2 mg/kg of mecamylamine, the ED_{50} for the discriminative effect of nicotine was reduced to 0.036 mg/kg. In contrast, in rats trained with nicotine plus 0.4 or 0.8 mg/kg of mecamylamine, nicotine did not acquire stimulus control over behavior. It might appear paradoxical that a small dose of mecamylamine reduced the ED_{50} for the discriminative effect of nicotine. However, that finding was predicted on the basis that the procedure was equivalent to training with a reduced dose of nicotine, a manipulation that robustly increased sensitivity to nicotine in many discrimination studies reviewed by Smith and Stolerman [71].

Subsequent experiments using drug mixture methodology aimed to determine whether pre-session effects of drugs could serve as discriminative stimuli in a procedure called trace conditioning [72]. Effects of agonists used as training drugs were terminated before sessions began by administering antagonists. Thus, injections of nicotine (20 minute pre-session) or vehicle preceded administration of mecamylamine (10 minute pre-session), so as to block effects of nicotine during training sessions. Similarly, injections of morphine (30 minute pre-session) preceded administration of naloxone (10 minute pre-session). These drug discriminations were acquired slowly to a final accuracy of only 70–75%. Extinction tests confirmed stimulus control by nicotine in the presence of mecamylamine and by morphine in the presence of naloxone. Stimulus control by pre-session drug states may have been weak due to the time elapsed between termination of the drug effects and training (trace conditioning).The possibility was also investigated that an additional drug could serve as a mediating stimulus that increased the strength of stimulus control by filling the temporal gap in the trace conditioning procedure [73]. In this study the injections of nicotine or saline were followed after 5 minutes by administration of midazolam as the putative mediating stimulus. The nicotine antagonist mecamylamine was administered 5 minutes after midazolam so as to block effects of nicotine during training sessions. Extinction tests showed midazolam

had to be present for the expression of stimulus control by nicotine. In control subjects trained with nicotine and midazolam but without mecamylamine, stimulus control by nicotine was not dependent upon the presence of midazolam. The results suggested that the discriminative effects of one drug can be mediated by the action of a second substance, a finding that was conceptualized in terms of "occasion setting" [74]. In occasion setting, one of two stimuli (called the feature stimulus) is postulated to acquire stimulus control by signaling reinforcement contingencies that will be in operation when a second stimulus is presented (the target stimulus). In the experiments of Stolerman and Mariathasan [73], nicotine may have served as a feature stimulus and midazolam as the target. Additional studies are needed to validate this concept; other studies on occasion setting with drugs have not explored its relevance to drug discrimination established with operant conditioning procedures.

H. ASSOCIATIVE PROCESSES

1. Overshadowing and Its Reversal

A review of early work pertaining to the role of overshadowing in the discrimination of compound stimuli involving drugs can be found in Järbe et al. [75]. *Overshadowing is shown by a weakening of the response to a stimulus after conditioning with that stimulus in compound with one or more additional stimulus elements*. For example, in a study by Järbe and Johansson [5], groups of rats were trained in a T-maze to discriminate the effects of the muscarinic antagonist Ditran on its own or in combination with the anticholinesterase physostigmine. It was suggested that physostigmine may have overshadowed Ditran, but it was difficult to distinguish overshadowing from a conventional pharmacological interaction between these substances. Subsequently, Garcha and Stolerman [22] noted preliminary evidence for overshadowing in relation to the discrimination of a mixture of nicotine plus midazolam, two drugs between which there was no reason to suspect an agonist-antagonist relationship. Comparable evidence for a mixture of amphetamine plus pentobarbital was obtained by Mariathasan et al. [23]. However, none of these investigations were designed primarily as studies of overshadowing, which was evidenced by comparisons between different publications or by sequential comparisons in the same rats, rather than by directly comparable between-group data from within an experiment.

Mariathasan and Stolerman [76] studied overshadowing by training different groups of rats with (–)-nicotine in compound with vehicle or midazolam, followed by full dose-response determinations in each group. It was found that training with midazolam in compound with nicotine weakened the discriminative effect of nicotine in a manner related to the dose of midazolam. A small dose of midazolam reduced the response to nicotine and a larger dose completely abolished it. These findings clearly indicated that the discriminative effects of midazolam overshadowed those of nicotine. A reciprocal influence of conditioning with nicotine on dose-response curves for midazolam was also seen. These drugs were thought not to interact pharmacologically such that in rats trained with midazolam alone, nicotine had no effect on the midazolam

dose-response curve whereas in rats trained with nicotine alone, midazolam had no effect on the nicotine dose-response curve [3, 76]. As described in Section G above, Mariathasan and Stolerman [70] examined the effects of training with nicotine in the presence of the nicotine antagonist mecamylamine. The data differed from those in the study of overshadowing in several ways, including retarded acquisition and a shift to the left of the dose-response curve for nicotine after training with nicotine plus a small dose of mecamylamine. It should be noted that Li et al. [18] obtained evidence suggesting that overshadowing is more likely to occur under two-choice procedures than under four-choice procedures where more response options are available.

Studies with exteroceptive stimuli revealed other aspects of overshadowing that may also be relevant for pharmacological stimuli. Matzel et al. [77] showed that an audible tone overshadowed a visual stimulus in a conditioned suppression procedure employing footshock as the unconditioned stimulus. However, after extinction of the response to the tone by presenting it repeatedly in the absence of footshock, there was a very substantial recovery of the response to the visual stimulus. Overshadowing may therefore be a failure to express a conditioned response, rather than a failure of acquisition [77, 78].

A parallel study was carried out with drugs which confirmed that after training for 60 sessions, midazolam overshadowed nicotine to the extent that the discriminative effect of nicotine seen in control rats trained with nicotine alone was abolished (Figure 10-7A). The discriminative response to midazolam in one group of mixture-trained rats was then partially devalued by means of an extinction procedure that weakened the

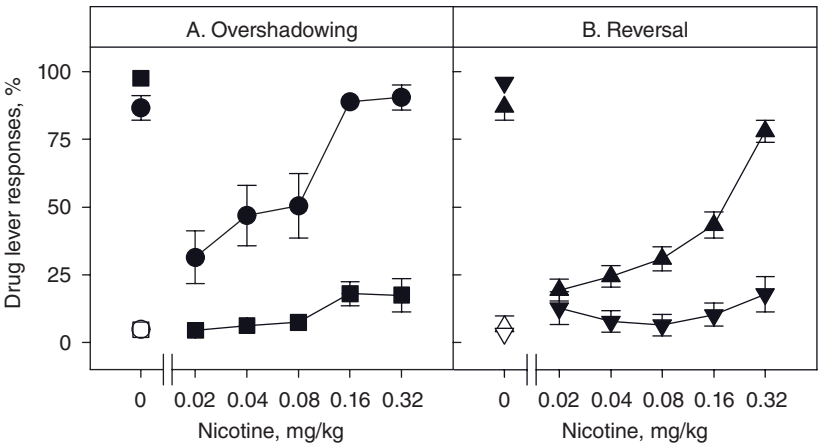

Figure 10-7. Discriminative stimulus effects of nicotine in (A) rats trained to discriminate nicotine from saline (●) or a mixture of nicotine plus midazolam from saline (■) and (B) rats initially trained to discriminate mixture from saline but for whom the response to midazolam was then either devalued (▲) or trained (▼). Points above zero on abscissae show results for tests with saline (open symbols) and training mixture (closed symbols). The same 0.32 mg/kg (SC) dose of each drug was used in all training and devaluation procedures. Other details as for Figure 10-4 (reproduced from [102]).

contingent relationship between drug and the response that was reinforced. The major finding was the restoration of the response to nicotine following the devaluation of stimulus control by midazolam, the overshadowing agent, without any further discrimination training with nicotine. Restoration, although only partial, was seen as a clear and unmistakable upward shift of the dose-response curve for nicotine (Figure 10-7B). Post-session injections of drugs were used to equate the pharmacological histories of the different groups of rats and the effects seen were therefore attributable to training with the drugs and not simply to repeated exposure to them. Nevertheless, the restored responses to nicotine were at most doses smaller than those in rats where it had never been overshadowed. The partial restoration of the overshadowed response may reflect the fact that the response to midazolam was only partially extinguished.

It was concluded that application of devaluation procedures in studies of the discriminative stimulus effects of single drugs with multiple effects may provide means for manipulating the characteristics of the discriminations obtained and for identifying individual elements of the drug-produced stimulus complex. It may be possible to use behavioral procedures such as devaluation to manipulate stimulus control by hypothesized individual elements of these stimulus complexes and thus clarify the mode of action of the drugs. The behavioral processes implicated in the restoration effect demonstrated yet another striking resemblance between the principles that govern the acquisition and extinction of stimulus control with drugs and with exteroceptive stimuli.

2. Associative Blocking

The impact of training sequence on discrimination of a mixture of two drugs has been investigated within the theoretical framework of associative blocking. Investigations involving exteroceptive events had defined ways in which the training sequence can influence stimulus control and patterns of generalization. *In some studies, stimulus control by one element of a compound stimulus was weakened if subjects had received prior training with the other element; a phenomenon that was called associative blocking* [21, 79]. The effects of prior training with a drug were first examined on the subsequent development of stimulus control by a compound stimulus comprising both the drug and an exteroceptive event. Järbe et al. [19] and Järbe and Johansson [80] showed that prior training with pentobarbital in a T-maze procedure attenuated the subsequent development of stimulus control by a visual stimulus that was trained in compound with the drug. Conversely, prior training with a visual stimulus attenuated the subsequent development of stimulus control by pentobarbital that was trained in compound with the visual stimulus.

Stolerman and White [81] initially trained rats for 60 sessions according to a two-lever, operant conditioning protocol; one group was trained with nicotine and another with midazolam. Three further groups of rats served as controls that were subjected to "sham" training in which dosing with saline, nicotine or midazolam was unrelated to contingencies of reinforcement. In the second phase of the study, all groups were then trained for 40 sessions to discriminate a mixture of nicotine plus midazolam from vehicle. Any subsequent differences between the groups in their performance could, therefore, be attributed to their different histories in the initial phase of training. All

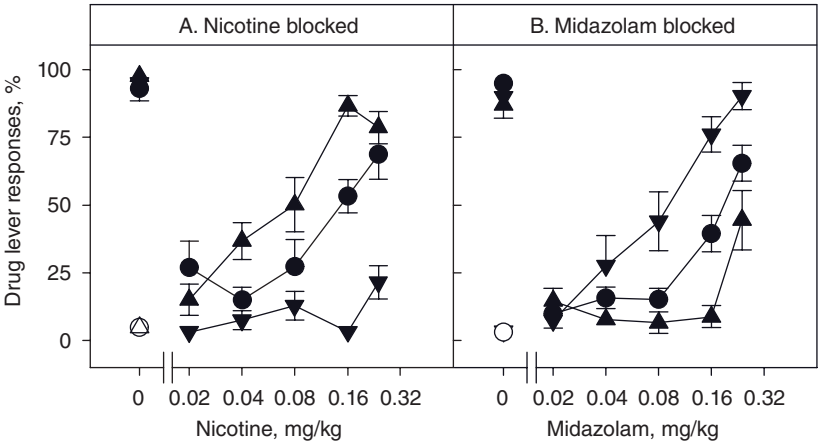

Figure 10-8. Dose-response curves showing associative blocking of nicotine (A) and midazolam (B) in three groups of rats trained to discriminate identical mixtures of the two drugs. Their previous histories included initial sham training (●), training to discriminate nicotine (0.4 mg/kg) from vehicle (▲) or midazolam (0.15 mg/kg) from vehicle (▼). Doses of nicotine (NIC) and midazolam (MDZ) are indicated by the dual scales on the abscissae. Points above zero on abscissae show responses to vehicle (open symbols) and the training mixture (closed symbols) for each group. Other details as for Figure 10-4 (redrawn from data of [81]).

groups acquired the mixture discrimination with similar accuracy (89–94% drug-appropriate responding after mixture as compared with 2–7% after saline). In the three groups subjected initially to "sham" training, there was partial generalization to the training doses of nicotine (45–53%) and midazolam (39–40%), each of which therefore contributed about equally to stimulus control by the mixture. However, the response to nicotine reached a maximum of 87% in the rats initially trained on nicotine, as compared with only 21% in initially midazolam-trained subjects (Figure 10-8A). Conversely, the response to midazolam reached a maximum of 87% in the rats initially trained on it, as compared with only 3% at the training dose in subjects initially trained on nicotine; this response was restored to 44% at a dose larger than that used for training (Figure 10-8B). Stolerman and White [81] interpreted the powerful and persistent effects of training sequence as examples of associative blocking. The use of sham-trained control groups that received matched drug treatments ruled out most non-associative interpretations of the findings.

I. INVESTIGATIONS ON THE ETHANOL CUE AS A COMPOUND STIMULUS

Several neurotransmitter systems have been implicated in responses to ethanol, making it a prime candidate for testing the applicability to single drugs of the rules governing stimulus control by drug mixtures. Discrimination studies suggested that ethanol pro-

duces a stimulus complex composed of distinct components mediated by different receptor systems, notably $GABA_A$, NMDA, and $5\text{-}HT_{1/2}$ receptors [63]. The contributions of these components varies according to the training dose of ethanol [63, 82, 83]; this may relate to variation in relative strength of drug mixture components when the absolute dose of some mixtures is varied (section E above). When ethanol is the training drug, barbiturates, benzodiazepines and NMDA antagonists generalize (e.g., [84, 85, 86]), which is compatible with the findings from most studies where the component drugs were tested in subjects trained to discriminate drug mixtures.

Strikingly asymmetrical generalization between ethanol and $GABA_A$-positive modulators, NMDA antagonists and 5-HT agonists is often seen. When barbiturates, benzodiazepines, or NMDA antagonists serve as the training stimulus, there is typically only partial generalization to ethanol [103, 52, 82, 87]. Similarly, the $5\text{-}HT_{1B/2C}$ agonist trifluoromethylphenylpiperazine (TFMPP) substituted for ethanol but ethanol did not substitute for TFMPP [83, 88, 89]. Thus, asymmetrical generalization has been found for all of the main neurotransmitter system thought to participate in ethanol discrimination; this is very different from results for studies of drug mixture discriminations where stimulus generalization from component drugs to mixtures is commonplace.

Manipulating the training paradigm has provided a partial solution to the problem of asymmetrical generalization with ethanol. Grant and colleagues used an approach that may be regarded as a three-choice extension of the AND-OR paradigm; a series of ethanol versus $GABA_A$ modulator versus water discriminations, or ethanol versus NMDA antagonist versus water discriminations was explored in attempts to isolate the different components of the ethanol cue [90, 91, 92]. Ethanol was conceptualized as taking the place of a drug mixture in AND-OR discrimination. Patterns of generalization were altered by using the three-choice procedure and the findings suggested that ethanol discrimination was not solely dependent upon either the positive modulation of $GABA_A$ receptors, or upon antagonism of NMDA receptors. For example, in an ethanol versus midazolam versus vehicle discrimination, the basis for ethanol discrimination was shifted towards cues distinct from those produced by midazolam [93].

Another approach has been to test for generalization to ethanol after training with mixtures of drugs acting on the diverse receptors thought to mediate the ethanol stimulus. Harrison et al. [50] showed that rats trained on a mixture of diazepam plus ketamine generalized almost fully to ethanol. Stolerman and Olufsen [39] took this idea further by showing that a training mixture of chlordiazepoxide plus dizocilpine generalized fully to ethanol (Figure 10-9). There was a similar result when the training mixture consisted of pentobarbital plus dizocilpine and this response was dependent upon the use of a training dose of pentobarbital that was relatively large compared with that of dizocilpine. Therefore, by training on specific mixtures of drugs it was possible to create a stimulus with greater similarity to that of ethanol than of the component drugs, and the problem of asymmetric generalization was to some extent overcome. Concepts of overshadowing and blocking derived from studies of exteroceptive stimuli and applied to drug mixtures have also been applied to the ethanol stimulus complex. There was evidence for overshadowing between the elements of the complex ethanol stimulus. The relative prominence of the $GABA_A$ component and the $5\text{-}HT_{1B/2C}$ component appeared to be greater at smaller training doses of ethanol, both when between-group

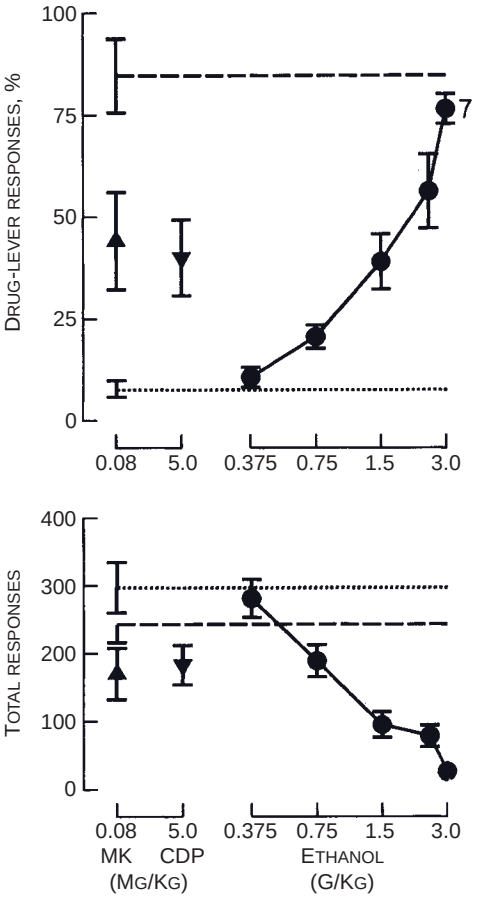

Figure 10-9. Generalization test results in rats trained to discriminate a mixture of dizocilpine (0.08 mg/kg IP) plus chlordiazepoxide (5.0 mg/kg SC) from vehicle (n = 9 except where indicated). Data are shown for ethanol administered by the intra-gastric route at doses of 0.375, 0.75, 1.5, 2.5, and 3.0 mg/kg (●) and for dizocilpine (MK, ▲) and chlordiazepoxide (CDP, ▼) at the doses used in the training mixture. Horizontal lines represent responses to vehicle (….) and training mixture (—). Other details as for Figure 10-4 (reproduced from [39]).

and within-subject designs were used [94]. These results were interpreted as evidence that at higher training doses of ethanol, other elements of the ethanol cue, such as NMDA antagonism, overshadowed its $GABA_A$ and $5\text{-}HT_{1B/2C}$ effects. However, an attempt to demonstrate associative blocking of components of the ethanol cue by prior training with a benzodiazepine or dizocilpine was unsuccessful [95]. Grant [63] went on to discuss implications for work with antagonists. For example, it was predicted that the ethanol cue would be more sensitive to blockade by either $GABA_A$ antagonists or NMDA agonists when trained against pentobarbital or a $5\text{-}HT_{1B/2C}$ antagonist, as com-

pared with a simple ethanol versus vehicle discrimination. This prediction remains to be tested.

J. DISCUSSION

1. Impact of Functional Models on Characteristics of Mixture Cues

The functional model upon which a discrimination is based has a profound impact upon patterns of generalization and antagonism. In the most commonly used AND discrimination procedure, individual drugs typically produce partial generalization when tested alone at the training doses and, in many cases, full generalization at larger doses (sections C and D). A mixture of morphine and dizocilpine is the only published exception to the general rule that under the AND discrimination model there is generalization to either component drug alone. In contrast, subjects were in all cases able to distinguish reliably between mixtures and component drugs if explicitly trained to do so in AND-OR and four-choice discrimination procedures.

The AND-OR procedure produced discriminations that were more specific pharmacologically than the AND discrimination procedure in terms of generalization profiles and sensitivity to antagonists. Nevertheless, the AND-OR procedure does not allow for distinctions between the stimuli produced by the two component drugs of the mixture and it does not allow for the identification of drugs that differ both from the training drugs and from the drug mixture. Four-choice procedures combine features of both AND and AND-OR procedures; the subject can make the mixture-associated response, or a response associated with either component of the mixture, or a vehicle-associated response. The four-choice procedure provides the greatest scope for detailed analysis of mixture cues; although it takes much longer to train, more information can be obtained from the trained subjects so the efficiency of data collection is more favorable than it might appear at first sight. A method to establish such discriminations within a shorter time period and in rodents would be a great asset for future research. In the remainder of this discussion, all statements refer to findings with AND discrimination procedures unless otherwise indicated.

2. Specificity of Stimuli Produced by Drug Mixtures

There is no evidence that training with mixtures extends the range of single compounds to which generalization occurs beyond that predicted from the characteristics of discriminations based on their component drugs (sections C and F above). Generalization to drug mixtures where either one or both component drugs were different from those in the training mixture was also examined. While some results were predictable for characteristics of discriminations based on the individual drugs used, others were not, even with the AND-discrimination procedure. It was difficult to find drug mixtures containing two novel components (“Dual substitution” tests) to which full generalization occurred and this was especially the case for rats trained in the AND-OR discrimination procedure (sections F.2 and F.3).

3. Alterations in Relative and Absolute Training Doses

Raising the relative dose of one drug in a training mixture increases the extent to which it contributes to stimulus control by the mixture (section E). Stimulus control by the other drug can weaken when the dose of it used for training is reduced; in some cases, stimulus control by a component can weaken even if its training dose is held constant, due to overshadowing by the other drug in the mixture. If the slopes of the dose-response curves for the separate drugs are different, altering the absolute amounts of drug mixtures in training may have complex effects that are not often recognized in discriminations of single drugs (section E).

4. Orderly Data with Antagonists

It is abundantly clear from studies with selective receptor antagonists that in the AND-discrimination procedure, blocking receptors for just one component of a drug mixture has only a small or even no detectable impact on discrimination of the mixture. In the only case where antagonism of an AND-OR discrimination was studied, an antagonist of either component drug blocked the response to the mixture (section G). Furthermore, very small doses of a mixture of the two antagonists produced complete blockade, an observation that needs to be extended to a wider range of drug mixtures to determine its generality. There appears to be potential value in mixture discrimination procedures for assessing pharmacotherapies proposed for treating the abuse of drug mixtures.

5. Importance of Associative Processes

Conditioning mechanisms play important roles in modulating responses to single drugs. In the past, such mechanisms were largely unrecognized as a potential basis for drug–drug interactions or for effects of subjects' previous history. Other relevant behavioral mechanisms arise from work on perceptual masking [96, 97, 98] and the rate-dependency hypothesis [99]. There are several instances where one drug in a mixture can weaken the response to the other drug through overshadowing (section H.1). This is distinct from pharmacological antagonism. It occurs with drugs that do not interact pharmacologically, and the characteristics of such discriminations differ from those based on a mixture of an agonist plus its antagonist. Furthermore, the discriminative effect of an overshadowed drug can be at least partially restored by extinguishing the response to the other agent, as is the case for overshadowing with exteroceptive stimuli. Overshadowing may occur to varying extents depending upon the drugs used and this aspect needs further investigation. Studies of drug mixture discriminations have also illuminated the role of the previous history of a subject as a determinant of the characteristics of the cue obtained. With just a few exceptions such as Li and McMillan [100], a quarter-century of drug discrimination research has taken little account of the impact of sequentially training different discriminations. The results of the experiments on associative blocking (section H.2) provide a specific mechanism that in some cases can explain the profound impact of behavioral-pharmacological history on the characteristics of drug-produced stimulus control [19, 42, 80].

Further investigations are needed to determine whether blocking and overshadowing are important in the real world or if they are merely phenomena that can be demonstrated under idealized laboratory conditions. Nevertheless, they are proposed as possibly relevant to clinically used drugs as well as to abused mixtures of drugs.

6. Relevance to Single Drugs with Multiple Effects

The most extensive body of relevant work is that relating to ethanol, which is thought to act on $GABA_A$, NMDA and $5\text{-}HT_{1/2}$ receptors. Investigations have found evidence for overshadowing among the component stimuli and weak or nonexistent blocking by receptor antagonists given singly. Three-choice, single-drug discrimination procedures did, to a considerable extent, facilitate identification of components of the ethanol cue. It was also found that stimuli produced by mixtures of drugs that resembled some of the component stimuli generalized to ethanol, thus overcoming the asymmetrical generalization frequently seen in cross-generalization tests with single drugs. However, an attempt to demonstrate associative blocking of components of the ethanol cue was not successful. From the foregoing it is apparent that attempts to apply findings from studies of drug mixtures to ethanol have been moderately successful and have facilitated the design of studies that clarify its mechanisms of action. The lack of total success may relate to the fact that knowledge of drug mixture discrimination is largely limited to binary mixtures, whereas the ethanol cue seems to have at least three components. It is also possible, even likely, that these receptor systems interact with each other in complex ways that have not yet been taken into account.

7. Conclusions

Almost all published studies on the discrimination of drug mixtures have shown clear and reproducible effects that can be understood by reference to known pharmacological and behavioral concepts. The approach has further potential in analyses of actions of abused and nonabused mixtures of psychoactive drugs and in the analysis of the mechanisms of action of single drugs that act through multiple neurotransmitter systems and receptors.

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11

MAKING THE RIGHT CHOICE: LESSONS FROM DRUG DISCRIMINATION FOR RESEARCH ON DRUG REINFORCEMENT AND DRUG SELF-ADMINISTRATION

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

Drug discrimination procedures have played a key role in the analysis of psychoactive drugs for more than 40 years. When properly implemented, these procedures are remarkable for their pharmacological sensitivity, selectivity, and flexibility. This chapter is founded on the proposition that these desirable attributes derive in large part from an almost exclusive use of concurrent-choice schedules of reinforcement, which facilitate dissociation of drug-induced discriminative stimulus effects from other drug effects. The remainder of this chapter will be divided into two general sections. The first section will review basic principles of operant conditioning (see also Chapter 2) and discuss application of these principles to drug discrimination and to a related field of psychopharmacology—the study of drug reinforcement using drug self-administration procedures. We conclude that choice procedures have been broadly and profitably applied to studies of drug discrimination but have been underutilized in studies of drug reinforcement. The second section will discuss strategies for the application of choice procedures to drug self-administration based on experience with these procedures in assays of drug discrimination.

A. OPERANT CONDITIONING TO STUDY THE STIMULUS PROPERTIES OF DRUGS

More than 70 years ago, B.F. Skinner described a three-term contingency of operant conditioning [1, 2], and this three-term contingency has played a key role in guiding the evolution of procedures used to study the stimulus properties of drugs. The three-term contingency can be diagrammed as follows:

$$S^D \rightarrow R \rightarrow S^C$$

where S^D designates a *discriminative stimulus*, R designates a *response* on the part of the organism, and S^C designates a *consequent stimulus*. The arrows specify the contingency that, in the presence of the discriminative stimulus S^D, performance of the response R will result in delivery of the consequent stimulus S^C. As a simple and common example from the animal laboratory, a food-restricted rat might be placed into an experimental chamber that contains a stimulus light, a response lever, and a food pellet dispenser. Contingencies can be programmed such that, if the stimulus light is illuminated (the discriminative stimulus), then depression of the response lever (the response) will result in delivery of a food pellet (the consequent stimulus). Conversely, if the stimulus light is not illuminated, then responding does not result in the delivery of food pellets. Under these conditions, subjects typically learn to respond when the discriminative stimulus is present. Consequent stimuli that increase responding leading

to their delivery are operationally defined as *reinforcers*, whereas stimuli that decrease responding leading to their delivery are termed *punishers*. The contingencies that relate discriminative stimuli, responses, and consequent stimuli are defined by the *schedule of reinforcement*. For example, under a fixed-ratio (FR) schedule, a fixed number of X responses in the presence of the S^D is required to produce delivery of the S^C (e.g., an FR 10 schedule would require 10 responses). Far more complex schedules of reinforcement are also possible [3; see also Chapter 3].

Behavioral procedures that employ some version of the three-term contingency have long been used to generate behavioral baselines for the study of drug effects, and an early finding was that drugs could not only modify behavior controlled by other stimuli, but could also function themselves as either the discriminative stimulus or the consequent stimulus in the three-term contingency [4–7]. Procedures in which drugs serve as the discriminative stimulus are termed *drug discrimination* procedures, and procedures in which drug delivery serves as the consequent stimulus are termed *drug self-administration* procedures. However, despite the common roots of drug discrimination and drug self-administration in operant methodology, these two families of procedures have evolved in very different ways. More specifically, they have evolved to rely on qualitatively different schedules of reinforcement. The development of drug discrimination will be considered first.

1. Drug Discrimination

In their simplest forms, operant conditioning procedures employ a single response option (e.g., pressing a single response lever), and the primary dependent measure is the rate at which that response is emitted. Using this simple approach, a drug discrimination procedure might establish contingencies such that responding produces reinforcement after drug administration but not after vehicle administration. Under these conditions, the subject might be expected to respond at high rates after drug administration but not after vehicle administration, and the primary measure of drug-induced discriminative stimulus effects would be the rate of responding. Such single-response experimental designs have occasionally been used in drug discrimination research [6–9], and this approach is closely aligned to experimental designs in a related field of research known as "State-Dependent Learning" [10, 11]. However, these designs pose intractable dilemmas for data interpretation. In particular, the primary dependent variable of response rate integrates multiple drug effects that include not only discriminative stimulus effects, but also other effects that might include (a) stimulant or depressant motor effects, (b) effects on information processing and stimulus control, or (c) effects on the reinforcing value of the consequent stimulus. The resulting challenge for data interpretation can be illustrated with a hypothetical example. Suppose a subject has been trained to discriminate Drug A from vehicle using a single-response procedure, such that response rates are high in the presence of Drug A and low in its absence. Subsequently, the subject receives Drug A after a pretreatment with Drug B, and the resulting response rates are low. Such a result might reflect antagonism of the discriminative stimulus effects of Drug A by Drug B; however, this result might also reflect other effects of Drug B such as motor suppression, impaired information processing,

or reduced value of the consequent stimulus. The inherent difficulty in disentangling discriminative stimulus effects from other drug effects is so widely appreciated that it has essentially eliminated the use of single-response approaches in studies of drug discrimination. Instead, investigators rapidly adopted choice procedures that proved far more powerful, and these choice procedures have come to dominate the field of drug discrimination research [5, 6, 12].

In choice procedures, subjects can emit at least two different types of responses reinforced under at least two concurrent schedules of reinforcement, and the primary dependent variable provides a measure of response allocation rather than response rate. In a typical example from modern drug discrimination research, subjects might behave in an experimental chamber equipped with two identical response levers located side by side on one wall. After drug administration, responding on only one lever produces the consequent stimulus (e.g., a food pellet) under some schedule of reinforcement (e.g., an FR 10 schedule). Conversely, after vehicle administration, responding only on the other lever produces the same consequent stimulus under the same schedule. Under these conditions, drug delivery sets the occasion for the location of responding, and once a subject has been trained, discriminative stimulus effects are inferred from measures of response allocation across the available response options. A drug might also influence overall response rate by producing other effects (e.g., effects on motor competence, information processing or reinforcer value); however, these other drug effects typically have little systematic impact on response allocation because the manipulanda, consequent stimuli, schedules of reinforcement, and rates and patterns of responding are virtually identical for all response options. To illustrate the advantage of choice procedures, consider again the hypothetical situation in which a subject is trained to discriminate Drug A from vehicle and then pretreated with Drug B. In a two-lever drug discrimination procedure, responding would occur on one lever after delivery of Drug A and on the other lever after delivery of vehicle. If Drug B antagonized the discriminative stimulus effects of Drug A, then Drug B would induce a reallocation of responding from the drug-associated lever to the vehicle-associated lever, but response rates would not necessarily be altered. Conversely, if Drug B degraded motor competence, information processing or value of the consequent stimulus, but did not alter sensitivity to the discriminative stimulus effects of Drug A, then overall response rates might decrease, but the allocation of responding would not necessarily be affected (i.e., any residual responding might still be directed primarily toward the drug-appropriate lever).

In summary, choice procedures generate dependent variables that permit dissociation of discriminative stimulus effects from other drug effects. More specifically, measures of response allocation can provide behavioral data on the discriminative stimulus effects of drugs, whereas measures of response rate can provide less differentiated data on other drug effects (see also Chapter 3). Moreover, choice procedures used to study drug discrimination in animals can be readily adapted to studies in humans, and virtually all human drug discrimination studies also use choice rather than single-response procedures [13–16]. This homology in procedure facilitates translational research and likely contributes to the excellent concordance between animal and human drug discrimination data. Overall, choice procedures have provided the methodological basis for development of sensitive, selective and flexible assays of drug discrimination in

both animals and humans, and the availability of these assays has contributed to the success of drug discrimination research during the last 40 years.

2. Drug Self-Administration

As noted above, the simplest operant conditioning procedures employ a single response option (e.g., pressing a single response lever), and the primary dependent measure is the rate of responding or the rate at which the consequent stimulus is delivered. Applying this formula to studies of drug reinforcement, a simple drug self-administration procedure might establish contingencies such that, in the presence of a discriminative stimulus (e.g., a visual stimulus), emission of a response produces delivery of a drug. The first published studies of intravenous drug self-administration used exactly this type of single-response procedure to examine morphine self-administration by morphine-dependent rats [17, 18]. Specifically, in the presence of a response lever (the discriminative stimulus), responding under a fixed-ratio schedule (FR 1 to FR 10) produced injections of morphine (0.1–10 mg/kg/injection). The primary dependent measures were the rates of drug delivery and the rates and patterns of responding.

Among other findings, these early studies revealed three phenomena that have been commonly observed in single-response procedures ever since. First, these studies demonstrated that intravenous morphine could maintain schedule-appropriate rates and patterns of responding leading to its delivery, indicating that morphine functioned as a reinforcer and produced reinforcing effects. This finding has since been extended to a wide range of other drugs, most of which have abuse potential in humans, and, as a result, drug self-administration procedures have been widely used to assess abuse liability [19]. Second, the procedure yielded a bitonic, "inverted U-shaped" dose-effect function relating the unit dose of morphine in each injection to measures of self-administration rate (either response rate or rate of injection delivery). Thus, maximal rates of self-administration were maintained by intermediate morphine doses, and lower rates were maintained not only by lower morphine doses, but also by higher doses. Why would rates of drug self-administration decrease as dose was increased above some optimal level? This question has vexed drug self-administration researchers for decades [4, 7, 20], but, as is often the case in other domains of pharmacology, the presence of bitonic dose-effect curves indicates that multiple and opposing drug effects are being integrated into a common dependent variable. In the case of drug self-administration, measures of self-administration rate can be influenced not only by reinforcing effects (which would have the effect of increasing response rates), but also by other effects of the self-administered drug that can either increase or decrease response rates (e.g., effects that improve or impair motor competence or information processing). For the remainder of this chapter, these other drug effects will be referred to collectively as "reinforcement-independent rate-altering effects" to distinguish them from reinforcing effects. A third and final finding of these early studies was that rates of morphine self-administration could be altered by treatment with other drugs. These effects were interpreted to suggest treatment effects on drug reinforcement (and by extension, to provide evidence regarding mechanisms of drug reinforcement). However, just as self-administration rates can be influenced by multiple effects of the

self-administered drug, so these rates can also be influenced by multiple effects of a treatment drug (or of any other manipulation, such as a lesion or genetic modification) [21, 22]. More specifically, treatments can alter rates of self-administration not only by changing the reinforcing effects of the self-administered drug, but also by changing the reinforcer-independent rate-altering effects of the self-administered drug, or by producing their own reinforcement-independent rate-altering effects. Overall, then, these early studies illustrated the promise of drug self-administration as a tool to study drug reinforcement, but they also provided a glimpse of the challenges to interpretation of rate-based measures generated by single-response procedures.

Drug self-administration research has flourished since the early 1960s, and techniques for intravenous drug self-administration were rapidly extended to studies with other drugs in other species of experimental subject (e.g., [4, 23–26]). However, while drug-discrimination research rapidly embraced choice procedures as the dominant experimental approach, drug reinforcement research has evolved along three divergent paths. One branch of the self-administration family tree has retained the use of single-response procedures while introducing schedules of reinforcement more demanding than the simple fixed-ratio schedules used by Weeks and colleagues. Table 11-1 summarizes some prominent examples of these single-response approaches, along with brief descriptions of their principal strengths and weaknesses. Studies using these approaches have shown that many drugs can maintain rates and patterns of responding similar to those maintained by nondrug reinforcers under a wide range of schedule conditions, and such findings have provided compelling support for the hypothesis that drugs can function as reinforcers [27]. However, these approaches have been less successful in generating dependent measures that reliably dissociate drug-induced reinforcing effects from reinforcement-independent rate-altering effects.

A second branch of the self-administration family tree has employed schedules of drug self-administration on one response lever, but has also incorporated rudimentary aspects of choice by introducing an "inactive" response option in addition to the "active" drug self-administration option. For example, in an early study to assess the reinforcing effects of cocaine, Pickens and Thompson [24] used a two-lever procedure in which responding on one "active" lever produced cocaine delivery under an FR 1 schedule, whereas responding on a second "inactive" lever had no scheduled consequences. Initially, responding was maintained exclusively on the active lever, and when the contingencies were reversed, rats rapidly reallocated their responding to the newly active lever. Many current studies continue to use an "inactive" manipulandum, and differential rates of responding on active and inactive manipulanda can be useful for evaluating the reinforcing effects of consequent stimuli associated with the active manipulandum. However, the value of this simple type of choice procedure is limited for at least two reasons. First, although "active/inactive-response" procedures technically employ a concurrent schedule capable of generating measures of response allocation and choice, such measures are rarely computed or reported. Rather, investigators more commonly report measures of response rate or reinforcement rate on the active manipulandum as if it were the only response option available, and such rate-based measures are vulnerable to all the reinforcement-independent rate-altering effects described above. Second, baseline response rates on the active and inactive manipulanda are usually very different, with rates on the inactive manipulandum being very low. As a result, data on

TABLE 11-1. Summary of single-response approaches that have been used in preclinical studies of drug reinforcement

Schedule of Reinforcement	Strengths	Weaknesses	Reference
Fixed-ratio schedules with long time outs	a	e,f	[96], [97]
Fixed-interval schedules	a	e,f	[98], [99]
Second-order schedules	a	e,f	[27], [100]
Progressive-ratio schedules	b	e,f	[96], [101]
Multiple schedules/Multiple groups	c	e,g	[21], [48]
Ratio schedules + behavioral economic analysis	d	h,i,j	[102], [103]

In this table and elsewhere in this chapter, the term "reinforcement-independent rate-altering effects" refers to drug effects other than reinforcing effects that might influence rates of drug self-administration. The approaches described below were developed largely in an attempt to either (a) reduce the impact of reinforcement-independent rate-altering drug effects and thereby reveal more clearly the impact of reinforcing effects on the primary dependent measure, or (b) provide strategies of data collection or analysis that might permit dissociation of reinforcing effects from reinforcement-independent rate-altering effects.

[a] Drug reinforcement alters the probability of behavior that *precedes* drug delivery, whereas many reinforcement-independent rate-altering drug effects (e.g., motor effects) alter the probability of behavior that *follows* drug delivery. By using long time-outs in ratio schedules, long intervals in interval schedules, or combinations of these approaches in second-order schedules, the effects of drug reinforcement on behavior preceding each injection can be retained, while reinforcement-independent rate-altering drug effects on behavior that follow each drug injection can be allowed to dissipate, thereby reducing their impact on overall rates of drug self-administration. The primary dependent variable is usually rate of responding or drug delivery, and changes in these rates are often interpreted as changes in drug reinforcement.

[b] In progressive-ratio schedules, the response requirements for sequential drug deliveries increase according to some algorithm until subjects either fail to respond or fail to complete the response requirement in a fixed period of time (a limited hold). The primary dependent measure is the "break point," defined as either the magnitude of the final ratio completed or the total number of drug doses delivered. Changes in "break point" are often interpreted as changes in drug reinforcement. Insofar as progressive ratio procedures permit relatively long intervals between injections, they serve to reduce the impact of reinforcement-independent rate-altering drug effects in much the same way as long time outs or intervals in ratio, interval and second-order schedules. The increasing ratio requirements also incorporate some strengths of "behavioral economic" strategies described below (see "d" below).

[c] In multiple schedules (for within-subject designs) or multiple groups (for between-subject designs), responding can be maintained by the self-administered drug of interest in one component of a multiple-component session or in one group, and by some other reinforcer (e.g., food) in a different component of a multiple component session or a different group. Importantly, subjects have access to only one reinforcer at any given time. The primary dependent variables are the rates of responding or reinforcement maintained by each of the two reinforcers. Selective changes in rates of drug self-administration without changes in rates maintained by the other reinforcer are often interpreted as changes in drug reinforcement. Nonselective changes in rates of responding or reinforcement maintained by both drug and the other reinforcer are often interpreted as evidence of reinforcement-independent rate-altering drug effects.

[d] "Behavioral economic" approaches to the design and interpretation of drug self-administration studies use variations on ratio schedules of reinforcement, and they seek to manipulate drug "price" as the independent variable and drug "consumption" as the principal dependent variable. Price is calculated as a fraction, with fixed-ratio response requirement in the numerator and unit dose in the denominator, and price can be manipulated by changing either of these components (e.g., price can be increased either by increasing the response requirement in the numerator or by reducing the unit dose in the denominator).

(*Continued*)

TABLE 11-1. (*Continued*)

Consumption is defined as the total intake during an experimental session. A graph with log price on the abscissa and log consumption on the ordinate yields a function known as a "demand curve," and demand curves typically display an accelerating negative slope. Thus, consumption is highest at the lowest price, and consumption decreases as price increases. The term "elasticity" is used to describe the sensitivity of consumption to increases in price, and various mathematical approaches have been used to quantify elasticity. In demand-curve analysis, nonspecific drug effects are thought to be revealed primarily by changes in maximal consumption at the lowest prices on the demand curve, whereas reinforcing drug effects are thought to be reflected by changes in elasticity.

[e] Although the impact of reinforcement-independent rate-altering effects of the self-administered drug is reduced, these procedures still often generate inverted U-shaped dose-effect curves with descending limbs. When self-administration dose-effect curves have a descending limb, then self-administration rates cannot be interpreted as a reliable indicator of drug reinforcement.

[f] A treatment drug or other manipulation (e.g., a lesion) can alter rates of drug self-administration by altering the reinforcing effects of the self-administered drug, altering reinforcement-independent rate-altering effects of the self-administered drug, or producing its own reinforcement-independent rate-altering effects. Consequently, treatment induced changes in self-administration rates do not provide a reliable indicator of changes in drug reinforcement.

[g] Manipulations can differentially alter response rates maintained by different consequent stimuli via mechanisms other than selective changes in the reinforcing effects of those stimuli. For example, two different reinforcers might differ not only in type, but also in relative magnitude (e.g., a relatively small drug dose and large food reinforcer), and manipulations are more likely to alter responding maintained by the smaller magnitude reinforcer.

[h] The principal independent variable in behavioral economics research is price, and a given price can be achieved with many different combinations of response requirement (in the numerator) and unit dose (in the denominator). For example, both FR 10/1 mg/kg/inj and FR 100/10 mg/kg/inj yield equivalent prices of "10" and would be expected to maintain equivalent consumption; however, this prediction holds over only a limited range of ratio requirements and unit doses (e.g., low drug doses might fail to maintain self-administration even at low response requirements, and high doses might produce toxic or lethal effects that limit consumption despite the apparently low price). As a result, the parameters actually used must be empirically determined.

[i] Behavioral economic approaches assume that reinforcement-independent rate-altering effects of a self-administered drug will be constant across all prices and will therefore not contribute to measures of elasticity; however, there is good reason to suspect that this is not the case. Rather, reinforcement-independent rate-altering effects are likely to be greatest when consumption is highest (when prices are low), and these effects should decline as consumption declines (when prices are high).

[j] The behavioral economic measure of drug reinforcement (elasticity) requires assessment of consumption across a broad range of prices, and as a result, this approach can be quite time consuming.

inactive responding are useful primarily for detecting reinforcement-independent rate-*increasing* effects. However, because inactive rates are already low, they are insensitive to reinforcer-independent rate-*decreasing* effects of experimental manipulations. This is a critical issue, because many studies are designed to evaluate the ability of experimental manipulations to decrease drug reinforcement as indicated by decreases in drug self-administration. For this type of study, procedures that use active and inactive manipulanda are little better than single-response procedures.

The third branch of the drug self-administration family tree has used concurrent schedules in which responding is maintained on two or more manipulanda by two or

more motivationally relevant consequent stimuli. For example, responding on one manipulandum might result in delivery of a particular drug dose, and responding on a different, concurrently available manipulandum might result in delivery of a different dose of the same drug, a different drug, or a qualitatively different consequent stimulus such as food. Under these conditions, the relative reinforcing effects of the drug in comparison to the alternative are inferred from measures of drug choice. As with any other procedure, drug delivery might also influence overall response rate by producing reinforcement-independent rate-altering effects; however, the impact of these other effects on drug choice can be controlled by appropriate use of manipulanda, discriminative stimuli, and schedules of reinforcement for the different response options. These are true choice procedures, and the challenges and opportunities associated with their use will be discussed more extensively in the next session. The key point to note here is that choice-based drug self-administration procedures mirror choice-based drug discrimination procedures in that they can generate measures of both response allocation and response rate that can be used to dissociate drug-induced stimulus effects from other drug effects. To illustrate this point, Figure 11-1 shows data from drug discrimination and drug self-administration studies with heroin in rhesus monkeys. The drug discrimination and drug self-administration choice procedures both generate measures of behavioral allocation that serve as measures of discriminative stimulus and reinforcing effects, respectively. Moreover, in both types of choice procedures, increasing heroin doses produced monotonic, dose-dependent increases in these measures of response allocation. The monotonicity of these dose-effect curves provides one source of evidence to suggest that choice-based measures of drug discrimination and reinforcement are not contaminated by other, opposing drug effects. Figure 11-1 also shows that drug discrimination and drug self-administration choice procedures generate measures of response rate that can be used to assess other rate-altering effects. In contrast, single-response drug self-administration procedures generate only rate-based measures, and these measures are influenced both by reinforcing effects and by reinforcement-independent rate-altering effects.

The value of choice measures has long been appreciated in studies of drug reinforcement. As noted above, the earliest studies of *intravenous* drug self-administration used single-response procedures, but these studies were predated by choice studies in which drug was delivered by other routes of administration. For example, Spragg evaluated choice between intramuscular morphine and fruit in morphine-dependent chimpanzees and demonstrated that choice was largely influenced by the state of morphine withdrawal (such that morphine withdrawal was associated with increased probability of morphine choice) [28]. Similarly, Nichols and colleagues established responding for oral morphine in rats and found that morphine withdrawal increased choice of morphine over water [29]. Intravenous drug delivery subsequently gained prominence in drug self-administration research because it promotes a rapid onset of drug action that facilitates learned associations between responding and drug delivery. However, the rise of intravenous drug self-administration was accompanied by a growing reliance on single-response and active/inactive-response procedures, perhaps because the limited lifespan of intravenous catheters selected for procedures that require the least initial training. Despite this trend, the use of choice persisted, especially in studies of oral

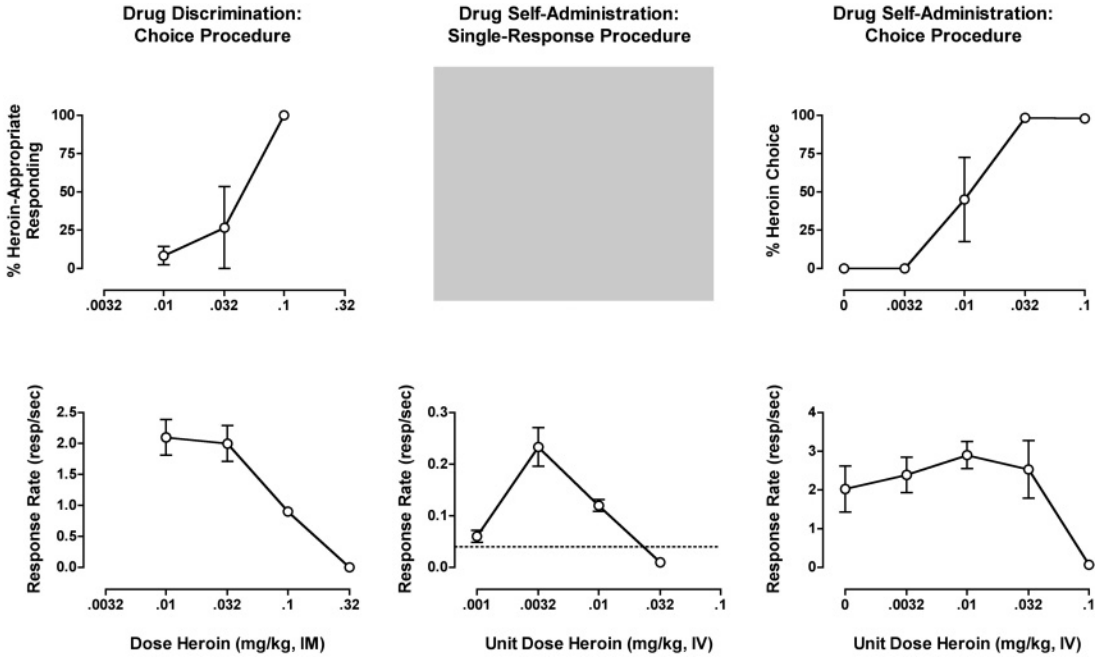

Figure 11-1. Illustrative data from drug discrimination and drug self-administration studies with heroin in rhesus monkeys. Each panel shows the relationship between drug dose on the abscissa and various measures of behavior on the ordinate. Left panels show data from a drug discrimination study in which three rhesus monkeys were trained to discriminate 0.1 mg/kg IM heroin from saline in a standard two-key, food-reinforced drug discrimination procedure [95]. The upper left panel shows a measure of behavioral allocation (% Heroin-Appropriate Responding), which is used to indicate drug-induced discriminative stimulus effects. The lower left panel shows Response Rate, which provides a measure of other rate-altering drug effects. The Center Panel shows data from a study in which three rhesus monkeys were trained to self-administer heroin in a single-response procedure (fixed-ratio 30/time out 60 second schedule) [47]. Single-response procedures generate measures only of response rate or reinforcement rate, and this figure shows the typical "inverted-U-shaped" dose-effect curve obtained in such procedures (dotted line indicates levels of responding maintained by vehicle). Note that single-response procedures do not provide measures of response allocation. Right panels show data from a drug self-administration choice procedure in which three rhesus monkeys could respond on one key for food pellets or on a separate, concurrently available key for heroin injections [47]. The upper right panel shows a measure of behavioral allocation (% Heroin Choice), which is used to indicate drug-induced reinforcing effects. Note that heroin produced a monotonic dose-dependent increase in this measure of drug reinforcement. The lower right panel shows Response Rate, which provides a measure of other rate-altering effects.

drug self-administration [4, 30] and in a small but steady series of intravenous drug self-administration studies (see below).

To illustrate the prevalence of single-response, active/inactive-response, and choice procedures in modern drug self-administration research, we conducted a literature search of PubMed on August 26, 2009, using the key words "Drug Self-Administration" to identify the 50 most recent preclinical studies. All manuscripts were published in 2009, nearly 50 years after the first report of intravenous drug self-administration. They described experiments conducted by multiple laboratories on three continents using four different species of subject to evaluate behavior maintained by 16 different drugs. Consequently, this sample provided a momentary but sweeping look at current practices. Of these 50 studies, only 8 (i.e., 16%) used choice procedures. Another 8 used single-response procedures, and 34 used active/inactive-response procedures. In contrast, a parallel search of the 50 most recent drug discrimination studies (key words "Discriminative Stimulus Effects") revealed that 92% (46 of 50) used choice procedures. This striking discrepancy suggests that there is substantial room for growth in the application of choice procedures to studies of drug reinforcement.

The discussion above has focused largely on the ability of choice procedures to generate dependent measures that permit dissociation of drug-induced stimulus effects from other, rate-altering effects. However, two other points are worthy of mention before proceeding. First, although choice procedures are sparingly used in *preclinical* studies of drug reinforcement, they have emerged as the standard approach in *clinical* studies [31, 32]. Consequently, increased preclinical use of choice procedures might facilitate translational research on drug reinforcement just as it facilitates translational research on drug discrimination. Second, scientific interest in drug reinforcement derives in large part from its presumed role in drug addiction, and drug addiction can be defined as a disorder of choice and behavioral allocation [33, 34]. Thus, addiction implies excessive drug choice at the expense of more adaptive behaviors. The factors that influence drug choice and contribute to addiction can be directly studied using choice procedures.

B. CHOICE PROCEDURES IN STUDIES OF DRUG REINFORCEMENT: LESSONS FROM DRUG DISCRIMINATION

Several outstanding reviews have summarized the influence of key independent variables on data obtained in drug discrimination experiments [5, 6, 35, 36]. The remainder of this chapter will consider analogous pharmacological, environmental and subject-related variables that either have been shown to operate or can be expected to operate in drug self-administration choice studies. Throughout this section, parallels will be drawn between the use of choice procedures in drug discrimination and drug self-administration research with the goal of applying experiences from the former to the latter. In addition, this section will focus on the use of choice procedures to study intravenous drug self-administration, and Table 11-2 summarizes the published literature. Although choice procedures can be and have been used with other routes of administration (especially the oral route), studies using the intravenous route

TABLE 11-2. Summary of published manuscripts reporting on IV drug self-administration under concurrent-choice schedules

Drug (dose in mg/kg/inj)	Alternative Reinforcer	Species	Main Effect Examined	Ref.
Cocaine (0.05–0.1)	Cocaine (0.013–0.8)	Rhesus	Effect of drug dose	[59]
Cocaine (0.05–0.1)	Cocaine (0.05–0.1)	Rhesus	Effect of schedule type	[58]
Cocaine (0.05–1.5)	Cocaine (0.1–1.5) Methylphenidate (0.075–0.07) Diethylpropion (0.5–1)	Rhesus	Effect of various pharmacological and environmental manipulations	[37]
Cocaine (0.05–1.5)	Methylphenidate (.075–.7)	Rhesus	Drug vs. drug preference	[38]
Cocaine (0.05 or 0.1)	Cocaine (0.013–0.8)	Rhesus	Effect of drug dose	[104]
Cocaine (0.1–0.75)	Cocaine (0.1–0.75)	Rhesus	Effect of punishment (electric shock)	[88]
Cocaine (0.3)	Food pellet	Rhesus	First study of cocaine vs. food choice	[86]
Cocaine (0.1–0.3)	Food pellet	Rhesus	Effect of chronic lithium treatment	[105]
Cocaine (0.05–0.3)	Food pellet	Rhesus	Effect of chronic antipsychotic treatment	[70]
Cocaine (0–0.1)	Procaine (0.4–1.6)	Rhesus	Drug vs. drug preference	[39]
Cocaine (0.05–0.2)	d,l-Cathinone (0.05–0.2)	Rhesus	Drug vs. drug preference	[44]
Cocaine (0.03–0.56) Procaine (1–10)	Food pellet	Rhesus	Effect of drug type and food reinforcer magnitude	[72]
Cocaine (0.03–1)	Food pellet	Rhesus	Effect of response requirement	[73]
Cocaine (0.03–1)	Food pellet	Rhesus	Effect of food availability conditions	[89]
Cocaine (0.025–0.1)	Cocaine (0.025–0.1)	Rhesus	Effect of schedule type	[76]
Cocaine (0.05–0.4)	Food pellet	Rhesus	Behavioral economic analysis of choice	[106]
Cocaine (0.05–0.2)	Food pellet	Rhesus	Behavioral economic analysis of choice	[107]
Cocaine (0.05–0.2)	Cocaine (0.05–0.2)	Rhesus	Effect of reinforcement probability	[108]
Cocaine (0.025–0.1)	Cocaine (0.025–0.1)	Rhesus	Application of generalized matching law	[77]

TABLE 11-2. (*Continued*)

Drug (dose in mg/kg/inj)	Alternative Reinforcer	Species	Main Effect Examined	Ref.
Cocaine (0.05) Alfentanil (0.001–0.004) Methohexital (0.25–0.5)	Cocaine (0.05) Alfentanil (0.001–0.004) Methohexital (0.25–0.5)	Rhesus	Application of generalized matching law	[78]
Cocaine (0.025–0.05)	Food pellet	Rhesus	Application of generalized matching law	[109]
Cocaine (0.01–0.03)	PTT (0.01–0.03)	Rhesus	Drug vs. drug preference	[48]
Cocaine (0.0032–0.32)	Food pellet	Rhesus	Effect of dose and cocaine pretreatment	[56]
Cocaine (0.0032–0.1)	Food pellet	Rhesus	Effect of various pharmacological and environmental manipulations	[54]
Cocaine (0.03–0.3)	Cocaine (0.03–0.3)	Rhesus	Effect of infusion delay	[49]
Cocaine (0.0032–0.1)	Food pellet	Rhesus	Effect of chronic kappa opioid treatment	[68]
Cocaine (0.0032–0.1)	Food pellet	Rhesus	Effect of chronic methadone treatment	[69]
Cocaine (0–0.1)	Food pellet	Rhesus	Reinstatement of cocaine choice by dopaminergic compounds	[110]
Cocaine (0.025–0.2)	Cocaine (0.025–0.2)	Rhesus	Effects of dose and schedule manipulations	[79]
Cocaine (0.003–0.3) Methylphenidate (.003–.1) Amphetamine (0.003–0.1) Atomoxetine (0.01–0.3) Desipramine (0.3–1)	Food pellet	Rhesus	Effects of drug type on drug vs. food choice	[45]
Cocaine (0.0032–0.1) Heroin (0.0032–0.1) Cocaine + Heroin	Food pellet	Rhesus	Effects of drug mixtures on drug choice	[46]
Cocaine (0.0032–0.1)	Food pellet	Rhesus	Effect of punishment (IV histamine)	[90]
Cocaine (0.003–0.03)	Food pellet	Cynomolgus	Effect of social hierarchy	[66]

(*Continued*)

TABLE 11-2. (*Continued*)

Drug (dose in mg/kg/inj)	Alternative Reinforcer	Species	Main Effect Examined	Ref.
Cocaine (0.003–0.03)	Food pellet	Cynomolgus	Effect of 8-OH-DPAT treatment	[111]
Cocaine (0.025–0.05)	Cocaine (0.025–0.05) Food pellet	Rhesus	Effect of reinforcement delay	[112]
Cocaine (0.01–0.03)	Remifentanil (0.0001–0.0003)	Rhesus	Behavioral economic analysis of choice	[75]
Cocaine (0.003–0.1)	Food pellet 10% Sweet Condensed milk	Rhesus Squirrel monkey	Effect of acute and chronic aripiprazole treatment	[65]
Cocaine (0.1–0.056) Remifentanil Methohexital (0.32)	Cocaine (0.1–0.056) Remifentanil (0.0001–0.00003) Methohexital (0.32)	Rhesus	Application of generalized matching law	[40]
Cocaine (0.0032–0.1)	Food pellet	Rhesus	Effect of exposure to and withdrawal from extended cocaine access	[63]
Cocaine (0.8)	Cocaine (0.267–2.4) SKF82958 (0.003–0.03) (+)-PHNO (0.001–0.01) SKF82958 + (+)-PHNO	Rat	Effect of drug mixtures on drug choice	[41]
Cocaine (0.267 or 0.8)	Nicotine (8–75)	Rat	Drug vs. drug preference	[42]
Cocaine (0.038–3)	Heroin (0.025–0.05) Cocaine + Heroin	Rat	Effect of drug mixtures on drug choice	[43]
Cocaine (0.25–1.5)	Saccharin or Sucrose	Rat	Effect of sweet solutions on drug choice	[113]
Cocaine (0–1)	Ensure liquid food	Rat	Effect of acute and chronic aripiprazole treatment	[57]
Cocaine (0.3–1)	Cocaine (0.3–1)	Rat	Effect of infusion rate	[50]
Cocaine (0.4)	Heroin (0.025)	Rat	Effect of home cage environment	[87]
Heroin (0.32–0.96)	Food pellet	Baboon	Effect of methadone, naloxone treatment	[83]
Heroin (0.055–0.83)	Food pellet ± Heroin (0.055–0.83)	Baboon	Various pharmacological and environmental manipulations	[91]

TABLE 11-2. (*Continued*)

Drug (dose in mg/kg/inj)	Alternative Reinforcer	Species	Main Effect Examined	Ref.
Heroin (0.32 or 1)	Food pellet	Baboon	Effect of morphine, naloxone, secobarbital	[67]
Heroin (0.0032–0.1) Heroin + SNC80	Food pellet	Rhesus	Effect of drug mixtures on drug choice	[47]
Heroin (0.0032–0.1)	Food pellet	Rhesus	Effect of methadone, buprenorphine, naltrexone treatment in non-dependent and opioid-dependent monkeys	[55]
Heroin (0.0032–0.1)	Food pellet	Rhesus	Effect of morphine, amphetamine, clonidine, antalarmin, norbinaltorphimine treatment in opioid-dependent monkeys	[64]
MDMA (0.03–0.3)	Food pellet	Rhesus	Effect of ambient temperature	[52]
MDMA (0.03–0.3)	Food pellet	Rhesus	Effect of thyroid hormone levels	[114]
Methamphetamine (0.06)	Food pellet	Rat	Drug vs. food preference	[115]
Nicotine (0.015)	Nicotine (0.015)	Rat	Effect of infusion rate	[51]

Columns show the primary drug option(s), the alternative reinforcer(s) (sometimes also a drug), the species in which studies were conducted, the primary effect examined in the study, and the reference. Numbers in parentheses show drug unit doses in mg/kg/injection.

predominate in the broader scope of drug self-administration research and stand to benefit the most from the incorporation of choice procedures. Finally, it should be noted that many of these variables have been manipulated in single-response and active/inactive-response drug self-administration procedures, but we propose that further studies using choice procedures will be valuable for two reasons. First, insofar as choice procedures facilitate dissociation of reinforcing and rate-altering effects of the self-administered drug, they should also facilitate interpretation of changes in drug choice produced by manipulation of pharmacological, environmental, or subject-related variables. Second, choice procedures explicitly introduce alternative reinforcers as options to the available drug. Features of alternative reinforcers and the contingencies that govern their availability provide a rich source of new variables that can be manipulated in studies designed to assess mechanisms of drug reinforcement and to evaluate strategies for treatment of drug addiction.

1. Pharmacological Variables

a. Pharmacodynamic Factors of Training and Test Drugs Pharmacodynamic factors include a drug's *affinity* in binding to its receptor(s) and *efficacy* in activating transduction mechanisms coupled to its receptor(s). Together, these factors contribute to a drug's pharmacological selectivity. In drug discrimination, pharmacodynamic traits of the training drug define the pharmacological boundaries of the discriminative stimulus, and test drugs typically substitute for the training drug only insofar as they share pharmacodynamic traits with the training drug. Pharmacodynamic factors also play a clear role in the reinforcing effects of drugs, but the role of this factor in intravenous drug choice has not been extensively examined. For example, Table 11-2 indicates that fewer than 20 drugs have been studied, with cocaine (45 of 56 studies) and heroin (9 of 56 studies) being the most extensively investigated. Most drugs have been examined in only one or two studies, and important classes of abused drugs (e.g., benzodiazepines, cannabinoids, and hallucinogens) have not been examined at all. Two general strategies have been used to manipulate pharmacodynamic factors in studies of drug choice. In one approach, a choice is provided between two drugs to provide a comparison of their relative reinforcing effects [37–44]. The second approach evaluates choice of different drugs relative to some common nonpharmacological referent reinforcer, usually food [45–47].

b. Pharmacokinetic Factors of Training or Test Drugs Pharmacokinetic factors include absorption, distribution, metabolism, and excretion, and these factors are critical determinants of drug time course and drug distribution to the central nervous system (CNS). In drug discrimination, distribution to the CNS appears to be necessary for a drug to produce discriminative stimulus effects, but drug time course appears to have little qualitative effect on drug-induced discriminative stimulus effects (i.e., rapid-onset, short-acting drugs can share discriminative stimulus effects with slower onset, longer-acting drugs). Only a few studies have used choice procedures to assess the role of pharmacokinetic factors on drug choice. For example, one study used a choice procedure to assess the reinforcing effects of the very long-acting dopamine transport blocker 2-ß-propanoyl-3-ß-(4-tolyl)-tropane (PTT) [48]. Choice procedures may be especially advantageous for evaluating such long-acting drugs, because in contrast to single-response or active/inactive-response procedures, choice procedures do not depend on long post-injection time outs to permit dissipation of reinforcement-independent rate-altering effects. Choice studies have also been used to evaluate the degree to which rate of onset influences drug reinforcement, and intriguingly, animals preferred faster infusion rates of cocaine but slower infusion rates of nicotine [49–51]. The presumed requirement for CNS distribution to maintain drug choice has not been examined for drugs from any class.

c. Dose of Training or Test Drugs In drug discrimination, the training dose of the training drug defines the magnitude of the discriminative stimulus, and this in turn may influence both the potency and the maximal effectiveness of substitution drugs. Similarly, in choice procedures, drug dose appears to contribute to the magnitude

of a reinforcing stimulus. Thus, in studies conducted to date, increases in drug dose almost always produce monotonic increases in choice of a reinforcing drug (e.g., cocaine or heroin) versus a nondrug alternative reinforcer (e.g., food) [52–57]. Moreover, in studies that provide a choice between lower and higher doses of a reinforcing drug, the higher dose is almost always preferred [37, 38, 58–60]. This dose-dependent and monotonic relationship between drug dose and drug choice represents a critical point of departure from single-response and active/inactive-response procedures, where inverted-U-shaped dose-effect curves predominate (see Figure 11-1). Moreover, the results from choice studies have provided compelling evidence to suggest that the descending limb in single-response or active/inactive-response self-administration procedures likely results from reinforcement-independent rate-decreasing effects rather than from a reduction in reinforcing effects or the emergence at high doses of aversive effects. It will be of interest to evaluate the degree to which this principle generalizes to other abused drugs such as nicotine.

d. Drug History Drug history describes any instance of drug exposure prior to a particular test session. Drug discrimination requires a history of drug exposure associated with training, and, as noted above, this history of training with a particular drug sets the pharmacological boundaries of the discriminative stimulus. However, as used here, drug history is also intended to describe the effects of acute or chronic drug pretreatments to assess their ability to modulate the stimulus effects of a given drug. As with drug discrimination, choice procedures also usually implement some period of training during which subjects are exposed to drugs and choice contingencies. The influence of different training regimens has received little attention, although availability of alternative reinforcers does appear to attenuate acquisition of drug self-administration [61, 62]. Choice procedures have been used more extensively to examine effects of acute or chronic drug pretreatments once choice of a particular drug has been established. For example, withdrawal from extended access to heroin self-administration produced somatic withdrawal signs and dramatically increased choice of heroin versus food, but withdrawal from a similar regimen of extended access to cocaine self-administration had little effect on choice of cocaine versus food [63, 64]. Other studies have used acute or chronic pharmacological pretreatments to examine mechanisms of cocaine or opioid reinforcement or to assess candidate medications for the treatment of cocaine or opioid addiction [54, 55, 57, 64–70]. A significant advantage of choice procedures for this type of study is that measures of drug choice permit dissociation of pretreatment effects on drug reinforcement from reinforcement-independent rate-altering effects. This advantage could be readily exploited in studies of choice for abused drugs other than opioids or cocaine.

2. Environmental Variables

One significant advantage of choice procedures is the opportunity they provide for systematic manipulation of key environmental variables that can robustly influence drug choice without directly modifying the pharmacology of the self-administered drug. Studies to evaluate effects of these non-pharmacological environmental variables can

have profound implications for drug abuse prevention and treatment, because they can provide critical insights into non-pharmacological factors that promote or retard drug choice.

a. Type and Magnitude of Alternative Consequent Stimulus In drug discrimination procedures, a discrimination is usually established between a drug dose and vehicle; however, discriminations can also be established between different doses of the same drug, between different drugs, or between drug and nondrug stimuli, and the structure of the comparison can influence the resulting discrimination. Just as the type and magnitude of the comparator can influence the discriminative stimulus effects of a drug, so the type and magnitude of the comparator can also influence drug choice in choice procedures. One theoretical principle of interest in choice studies is that two different consequent stimuli A and B can function as substitutes, independent commodities or complements depending on the degree to which a change in consumption of A is associated with an opposing change, no change, or a similar change in consumption of B, respectively [71]. Drug choice studies have typically used alternative reinforcers that function as substitutes, such that changes in drug self-administration are associated with a reciprocal change in consumption of the alternative. For example, many studies have established a choice between cocaine and food, and manipulations that alter cocaine choice often produce a reciprocal and opposing change in food choice (e.g., decreases in cocaine choice are associated with increases in food choice [54, 72, 73]). However, the extent to which various drugs and other consequent stimuli might function as substitutes, independent commodities or complements has not been widely studied. In view of the extensive and growing use of alternative reinforcers and contingency management strategies in the clinical treatment of drug addiction [74], there is clearly a need for more extensive preclinical research to identify conditions under which safe alternative reinforcers might be most effective in reducing drug choice. For example, it might be of interest to assess the degree to which nonfood, nondrug positive reinforcers (e.g., response-produced access to exercise or social interactions) or negative reinforcers (e.g., avoidance or termination of electrical stimulation) might modulate drug choice.

b. Schedules of Reinforcement The influence of schedule conditions on drug discrimination has received limited study, and although several different types of schedules have been used, most drug discrimination studies use concurrent fixed-ratio schedules (see also Chapter 3). Drug choice procedures have also relied primarily on concurrent fixed-ratio schedules, and manipulation of these schedules has provided insight into the remarkable degree to which the contingencies for an alternative reinforcer can influence drug choice. For example, when cocaine injections and food pellets were available under concurrent fixed-ratio schedules, cocaine choice could be enhanced either by (a) decreasing the response requirement for cocaine injections or (b) increasing the response requirement for food delivery [54, 73].The reciprocal also held true in that drug choice could be reduced either by (a) increasing the response requirement for cocaine or (b) decreasing the response requirement for food. Similar results were also obtained in studies of choice between cocaine and the opioid agonist remifentanil

[75]. Thus, cocaine self-administration could be strongly influenced not only by contingencies that governed its own availability, but also by contingencies that governed the availability of an alternative. (Note that these results depended on the ability of the alternative reinforcer to function as a substitute for cocaine rather than as an independent commodity or a complement to cocaine.)

A weakness of concurrent fixed-ratio schedules is their tendency to generate quantal choice, which limits sensitivity to graded differences in the reinforcing strength of two consequent stimuli. As a result, many studies have also used concurrent variable- or random-interval schedules in an effort to generate more graded levels of choice and more precise assessments of differences in reinforcing strength between reinforcers [40]. More generally, studies with variable- or random interval schedules have been used to demonstrate that drug self-administration adheres well to predictions of the matching law, which posits that the allocation of behavior between two response options will match the frequency of reinforcement associated with those options [58, 76–79]. The matching law was originally developed to describe patterns of food-maintained responding under concurrent schedules [80, 81]. The ability of the matching law to also describe drug self-administration has contributed to the more extensive body of evidence indicating that responding maintained by abused drugs is sensitive to many of the same variables that govern responding maintained by nondrug reinforcers.

In drug discrimination procedures, tandem variable-interval fixed-ratio schedules have been suggested to generate discriminations as robust as those generated by fixed-ratio schedules but with more graded levels of drug-appropriate responding characteristic of variable-interval schedules [6, 82]. The utility of tandem schedules for studies of intravenous drug choice has not been assessed but might warrant study.

c. Discriminative Stimuli Insofar as studies of drug choice employ the three-term contingency, they necessarily include discriminative stimuli associated with the different response options. The role of discriminative stimuli in modulating drug choice has not been systematically examined, but several different approaches have been used, and each approach has strengths and weaknesses. In the most commonly used approach, the discriminative stimuli are similar for the available response options (e.g., similar colored stimulus lights; e.g., [50, 52]). By minimizing differences between discriminative stimuli, this approach minimizes the degree to which choice in determined by discriminative stimuli and maximizes the degree to which choice is determined by differences in the reinforcing strength of the consequent stimuli. However, this approach requires a relatively long period of experience with any change in choice options before behavior stabilizes and choice can be assessed. In a second approach, the consequent stimuli are also arranged as explicit discriminative stimuli by introducing them via noncontingent delivery (“priming”) or required contingent delivery (“sampling”) prior to choice components [56, 57]. This approach accelerates stability of choice, but also increases the role of discriminative stimuli as determinants of choice. In a third approach, different exteroceptive stimuli are associated with the different consequent stimuli [54, 83]. This approach further accelerates changes in choice following changes in choice options, and it may also align with the natural environment of drug use, wherein different discriminative stimuli (e.g., signs, labels, packaging, etc.) reliably predict

availability of different consequent stimuli. However, it also increases the influence of discriminative stimuli as determinants of choice.

d. Other Environmental Variables As an example of research to evaluate effects of other environmental variables on drug discrimination, it was found that restraint or social-defeat stress substituted for the discriminative stimulus effects of cocaine and/or amphetamine in some rats [84, 85]. Similarly, a growing body of research has addressed the degree to which environmental variables other than those described above might alter drug choice. To date, the environmental manipulations examined have included noncontingent delivery of an alternative food reinforcer, food deprivation, punishment of drug choice or of choice of an alternative using electric shock or intravenous histamine injections, reinforcement of drug choice by delivering other reinforcers in addition to the drug, changes in ambient temperature, social rank in group-housed subjects, and variations in housing conditions [46, 52, 54, 66, 86–91]. Similar studies on these and other environmental variables will play a key role in future research to identify environmental mechanisms that may differentially affect the reinforcing strength of drugs and underlie vulnerability to or protection from drug addiction.

3. Subject-Related Variables

Subject-related variables include age and genotype (species, strain, sex, polymorphisms, knockout or knockin manipulations) as well as other types of manipulations (e.g., lesions) that produce relatively permanent changes in the experimental subject. Subject-related variables have a profound influence on many drug effects, and these variables are also thought to play an important role in vulnerability to drug abuse (e.g., [92]). Some drug discrimination studies have explicitly manipulated subject-related variables (e.g., [93, 94]; see also Chapter 3), and more generally, the discriminative stimulus effects of drugs have been demonstrated in many species and strains of subject. Studies of intravenous drug choice have also been conducted in various species including rats, squirrel monkeys, cynomolgus and rhesus macaques, and baboons [41, 58, 65, 66, 83]. However, there is substantial opportunity for more systematic research on the role of these and other subject-related variables. Moreover, drug choice studies can be expected to contribute important insights that might not be apparent from single-response or active/inactive-response procedures. Specifically, as has been emphasized repeatedly above, drug choice is strongly determined by factors that influence the reinforcing strength of alternative reinforcers. Consequently, it should be anticipated that some subject-related factors would have profound effects on drug choice by modulating the reinforcing strength of alternative reinforcers while producing little or no direct changes in the reinforcing strength of the drug.

C. SUMMARY

Drug discrimination has emerged as a powerful family of procedures in psychopharmacology. The extraordinary utility of drug discrimination derives in large part from

its almost exclusive use of concurrent-choice schedules to generate a dependent measure (percent drug-appropriate responding) that provides a rate-independent measure of drug-induced discriminative stimulus effects. Studies of drug self-administration have been much slower to adopt concurrent-choice schedules; however, this chapter has argued that choice schedules can also be useful in preclinical research on drug reinforcement. Specifically, choice schedules can facilitate data interpretation by providing a rate-independent measure of drug reinforcement, improve concordance between preclinical and clinical studies in translational research, and provide experimental access to key independent variables that influence drug choice and drug addiction in natural environments.

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12

INHALANT DRUG DISCRIMINATION: METHODOLOGY, LITERATURE REVIEW AND FUTURE DIRECTIONS

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

A. INTRODUCTION

The first drug discrimination studies with abused inhalants were not begun until the mid-1980s, many years after drug discrimination methods had been used to study nearly every other class of abused drugs. One of the reasons for this was the perceived difficulty in arranging controlled exposures of animals performing operant behavior. The methods that have been developed since that time to overcome the technical challenges associated with inhalant studies are detailed later in this chapter. Another reason that inhalants were not examined earlier was probably the perception that these compounds produced "nonspecific" effects that would not be compatible with the sophisticated assessments we had come to expect for drug classes such as, for example, opioids, GABAergics, stimulants, etc. Nonetheless, there were many unanswered questions about the abuse-related effects of abused inhalants that seemed to lend themselves to study using drug discrimination procedures. These questions included:

- What is the nature of inhalant intoxication?
- Do inhalants produce an intoxication that resembles those for any other known classes of drugs of abuse or are they unique?
- Do all inhalants produce qualitatively similar intoxications?
- What is the neural bases for inhalant intoxication?
- Do inhalants differ in abuse liability, and if so, can a scientific basis be developed for recommending reformulation of abused products with components with less abuse liability?

Before discussing inhalant discrimination methodology and the current state of knowledge regarding their discriminative stimulus effects, some background is appropriate. The term *inhalant* is a generic classification for a diverse group of abused volatile or gaseous compounds with widely differing chemical structures. These compounds are present in a countless variety of readily accessible household products including: gasoline, adhesives, spot removers, spray paint, paint thinners, aerosol dusters, shoe polish, and varnishes to name just a few examples. While some inhalants undoubtedly have common behavioral and neurochemical actions, inhalants as a whole are unique among drug classes in that they are defined not by their effects but rather solely by their route of administration. One could easily argue that the lack of scientific basis inherent in this *prima facie* approach alone has greatly impeded our understanding of these compounds.

Inhalants are arguably the most poorly understood of all classes of abused drugs for a number of other reasons. A persistent underappreciation in the research community for the magnitude of the societal consequences of inhalant abuse is likely a major factor in the paucity of research in the area. The 2008 National Survey on Drug Use and Health estimates that over 22 million persons in the United States have ever used inhalants and 640,000 had used inhalants in the last month. Both of these statistics were greater than those reported for heroin, crack cocaine, methamphetamine, and several other reported illicit drug categories. In terms of drug discrimination research,

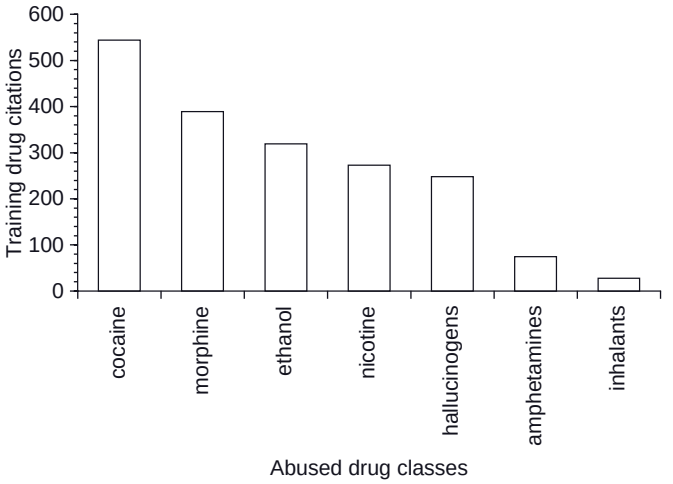

Figure 12-1. Total number of citations reporting the training of representative abused drugs using the drug discrimination procedure.

Figure 12-1 graphically illustrates the number of drug discrimination citations, including published papers and scientific conference abstracts, for a variety of training drugs or training drug classes (Drugrefs.org). Among these, cocaine has been trained the most frequently with 544 citations. In contrast, inhalants rank lowest with only 28 total citations. This figure includes 8 citations of inhalants as training drugs and, unlike the other drugs shown in the figure, also includes an additional 20 citations which used inhalants as cross-test drugs.

Laying aside the issues of the polyglot classification scheme for inhalants and insufficient perception of the severity of the problem, a more easily addressable potential cause for the limited number of studies examining the behavioral effects of abused inhalants are the perceived technical difficulties associated with these studies. One major focus of the present chapter will be to outline the challenges which have been encountered in conducting drug discrimination studies with inhalants as well as the solutions developed to overcome these challenges. While clearly of relevance to any researcher that wishes to explore inhalants as discriminative stimuli, the techniques used for studying inhalants in drug discrimination experiments are easily adaptable to other types of behavioral paradigms with vapors and gasses, indeed many of them were adapted for drug discrimination studies after first being developed for other uses. The second focus of the present chapter is to review the available literature examining inhalants as discriminative stimuli and draw some conclusions from the available data.

1. Classification of Inhalants

Given the shear number of potentially abusable inhalants, some meaningful framework is clearly necessary to categorize these compounds. There are a number of metrics by which one could classify inhalants [1]. Perhaps the most meaningful scheme, at least

as it relates to drug discrimination research, is to group inhalants according to their pharmacological properties. Unfortunately, the number of inhalants for which their pharmacological effects have been fully or even partially characterized is exceedingly small. In our laboratory, due to the absence of good pharmacological data, we have instead generally utilized both chemical family as well as product type to subdivide these chemicals into general categories.

Inhalants can broadly be classified as either being volatile vapors or gasses. The most prominent groups of abused inhalant vapors are 1) motor fuels, 2) volatile hydrocarbon solvents, and 3) inhalant anesthetics. Motor fuels are complex mixtures of dozens of petroleum hydrocarbons. For instance, gasoline contains various proportions of paraffins, naphthenes, and olefins as well as aromatic hydrocarbons like benzene, toluene, and xylenes. While abuse of motor fuel has been reported [2], the complex nature of fuel vapors makes them poorly suited to drug discrimination studies as interpreting data from such experiments would be exceedingly difficult. Volatile aromatic hydrocarbon solvents present in paint and lacquer thinners, spot removers, glues and other products are also often abused [3, 4]. These products can contain mixtures of multiple chemicals but often have a single predominant volatile constituent, such as toluene. While less common than they once were due to an international treaty phasing out use of some of these compounds in developed but not underdeveloped countries, chlorinated hydrocarbons, represented by compounds such as 1,1,1-trichloroethane (TCE), perchloroethylene, and trichloroethylene also have a record of abuse [5–8]. The final subgroup of abused volatile compounds are the volatile anesthetics. The prototypic member of this class is diethyl ether. Since ether is no longer used clinically it's abuse is now rare [9]. However, the replacement of ether by nonexplosive agents such as the halogenated alkane, halothane, and more recently halogenated ethers such as isoflurane, sevoflurane, desflurane, and methoxyflurane and has led to reports of abuse of these compounds [10–13].

Gasses represent the other primary category of abused inhalant compounds. The primary abused inhalant gas is nitrous oxide. Compounds that are liquefied under pressure but gasses at atmospheric pressure, such as spray can propellants and butane, could be classified as gasses as well but are generally referred to as vapors. Although nitrous oxide is subject to abuse there have been only two published studies with nitrous oxide that are relevant to drug discrimination [14, 15]. Both of these were conducted in humans using subjective effects questionnaires.

The majority of published drug discrimination research has involved volatile vapors. Therefore, for simplicity, unless otherwise noted, in the remainder of this chapter "inhalant" will generally be used in reference to volatile vapors.

B. INHALANT EXPOSURE METHODOLOGY

In order to provide a review of the general methodology used in the studies reviewed later in this chapter as well as guide researchers who themselves may wish to conduct behavioral studies with inhalants, a fairly detailed discussion of exposure methods is useful. Inhalant exposures can be carried out using either static or dynamic exposure

systems. A static system is completely sealed whereas a dynamic exposure system is constantly introducing fresh chamber atmosphere and exhausting stale atmosphere. Each type of apparatus has distinct advantages and disadvantages. The choice of one method over another is generally based on desired exposure parameters and the physical characteristics of the inhalant of interest. The basic components of both systems as well as their advantages and disadvantages are outlined briefly below.

1. Static Exposure Systems

A static inhalant exposure system is most useful for examining inhalants that originate as volatile liquids. Since our laboratory has been primarily concerned with examining solvents and volatile anesthetics we have utilized static exposure systems for the majority of our inhalant discrimination studies. In its most simplistic form, a static inhalant exposure apparatus consists of a reasonably airtight vessel in which an inhalant vapor can be generated and contained. For obvious reasons the exposure system must be designed in tandem with an effective means of evacuating waste vapors after the termination of the exposure period. To accomplish the latter goal, all of our static exposure systems are constructed in dimensions that allow them to be contained under a standard laboratory chemical fume hood. Decontamination of the chamber is then simply a matter of removing the lid and allowing the vapors to dissipate. Compressed air can be used to purge the chamber if the system is to be rapidly reused. In laboratories lacking a suitable fume hood, the evacuation of inhalant vapors is more complex and beyond the scope of this chapter.

When designing a static exposure apparatus it is important that the exposure vessel be large enough to contain the research subject or subjects as well as sufficient breathable air to prevent buildup of carbon dioxide and hypoxia during the vapor exposure. As one might surmise, a significant disadvantage of a static exposure apparatus is the fact that lengthy exposures and/or larger subjects require larger exposure vessels. We have exclusively used mice for our inhalant discrimination studies and generally limit duration of exposure to no more than 20 minutes. These conditions permit the use of modestly sized exposure chambers. Static chambers sufficiently large to permit exposing rats for similar short periods of time may be somewhat greater in dimensions but are still readily constructed. Extended durations of exposure in a static system may necessitate control testing with an oxygen analyzer to ensure the presence of a sufficiently high quality air supply.

Static exposure chambers can be manufactured from a variety of materials including glass, stainless steel, and plastics such as acrylic and polycarbonate. Glass and stainless steel are impervious to the effects of most solvent-based inhalants, whereas commonly available plastics are damaged by direct contact with solvents. However, in practice, the concentrations of inhalant vapors used in behavioral studies appear to have little effect on acrylic, even after many years of daily exposure, making it an excellent material from which to construct exposure chamber components.

The static chambers we use in our laboratory are based on commercially available, flat bottom, cylindrical, Pyrex glass jars. Jar volume varies with the manufacturer but are generally in the range of 25–27 cubic liters. A jar of 27 cubic liters provides more

than adequate volume to permit the simultaneous exposure of several mice for 10 to 20 minutes. Exposing multiple subjects at once can be accomplished by first placing the individual mice into smaller, ventilated containers. Several of these containers can then be inserted into the exposure chamber. For holding individual mice, stainless steel confectionary sugar or condiment shakers with wire mesh lids are an excellent, ready-made choice. In our static systems the lip of each jar is fitted with a closed-cell foam rubber gasket to ensure an airtight seal. A 3/8" thick acrylic lid is used as a cover to seal the open end of the jar. A fan mounted to the acrylic lid speeds the volatilization of inhalants and helps maintain a homogenous vapor concentration in the chamber. Small, inexpensive, AC fan motors in the 1/30 hp range are more than adequate for this purpose. Although the behavioral effects of many flammable volatile compounds are exhibited at concentrations below their lower explosive limit, it is nonetheless advisable that the fan motor be attached to the exterior of the chamber lid to prevent a spark from producing combustion. To accomplish this, the fan motor's drive shaft extends through a sealed bearing in the lid into the exposure chamber where it is attached to a small fan blade. Directly below the fan blade is a suspended wire mesh platform. Porous paper, that is, standard laboratory filter paper, is secured on the platform and serves as an absorbent surface onto which a liquid inhalant can be injected via a stoppered port in the chamber lid. Because many liquid inhalants have a tendency to dissolve or at least chemically interact with plastic syringes, the best means to measure and deliver the appropriate amount of liquid solvent onto the filter paper is with a gastight glass syringe fitted with a long blunt needle. Figure 12-2 shows one of the static exposure

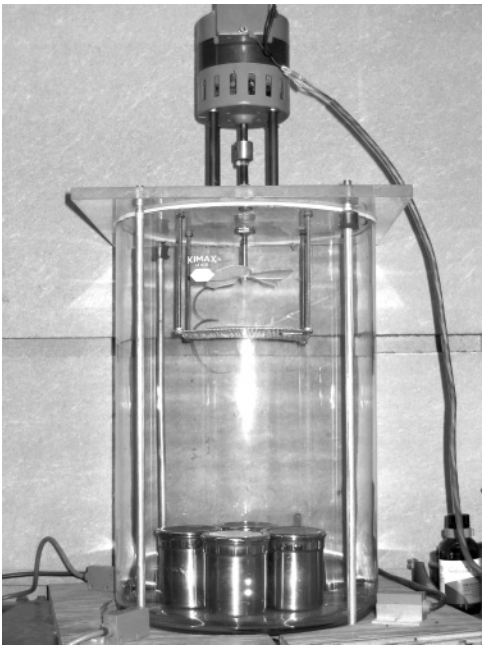

Figure 12-2. Static inhalant vapor exposure chamber.

$$C_{ppm} = \frac{26.5 \times 10^6 \ {}^{V}L \ {}^{\rho}L}{{}^{V}D M}$$

Figure 12-3. Ideal gas law equation solving for vapor concentrations at standard laboratory pressure and temperature.

chambers used in our laboratory. The motor, fan blade, and wire mesh platform are visible at the top of the chamber. The stainless steel individual animal containment jars can be seen in the bottom of the tank.

Static exposure chambers offer a number of distinct advantages over dynamic systems. Static systems are simple, durable, and can be constructed for as little as a few hundred dollars. While these are all desirable, perhaps the most important advantage of a static system is the ease with which exposure chamber vapor concentrations can be calculated. Since a static exposure chamber is of a fixed volume, the Ideal Gas Law can be used to determine the concentration of an inhalant vapor in the exposure chamber based on the introduction of a known amount of volatile liquid inhalant. The most general form of the Ideal Gas Law accounts for a number of environmental variables that are not of concern in inhalant exposure studies. In particular, minor daily fluctuations from standard laboratory temperature and atmospheric pressure have a negligible impact on calculated versus actual inhalant vapor concentrations at pharmacologically relevant levels. Assuming standard laboratory temperature and pressure allows the derivation of the Ideal Gas Law into the formula shown in Figure 12-3, where ${}^{V}L$ is the volume of inhalant liquid in ml, ${}^{\rho}L$ is the inhalant density, ${}^{V}D$ is the inhalant chamber volume in liters and M is the molecular weight of the inhalant [16].

Using the above formula it is trivial to determine the volume of volatile liquid inhalant which must be introduced into an exposure chamber to produce a given parts per million vapor concentration. In our laboratory we have converted this formula into a simple Excel spreadsheet with user-defined variables for exposure chamber size, inhalant molecular mass, and inhalant density. A major caveat of using this approach is that the inhalant under study must be sufficiently volatile. Fortunately, most abused inhalants volatilize fairly quickly and completely at the concentrations which are necessary to produce overt acute behavioral effects. Inhalants with lower volatility may require an excessively long period of time to convert to vapor or might even require heating to produce appropriate vapor concentrations. In both of these latter cases, a static system would not be an appropriate exposure apparatus.

2. Dynamic Exposure Systems

In situations in which a static vapor exposure system is not appropriate, a dynamic system can be constructed. A detailed discussion of the construction of a dynamic vapor exposure system would itself take up an entire book chapter or more and good descriptions are already available [16–18]. In brief, the basic components of a dynamic exposure system consist of a breathable compressed air source, a vessel containing liquid

inhalant though which clean air can be bubbled to produce vapor, metering systems to control the flow of clean and solvent laden air, the exposure chamber itself, and sufficient tubes and valves to connect these component. In a dynamic vapor exposure system, accurately and precisely controlling system pressure and flow rates is essential since alterations of these variables will determine inhalant vapor concentration. Some mechanism must also be incorporated to measure inhalant vapor concentrations, preferably in a real-time manner. The measurement of inhalant exposure concentrations will be explored in a subsequent section of this chapter.

The primary advantage of a dynamic exposure system over a static apparatus is that the duration of inhalant exposure is not limited by the amount of breathable air within the exposure chamber. For this reason, dynamic exposure systems have been used fairly extensively as a means of inducing tolerance to both inhalants and ethanol vapors [17, 19]. Dynamic systems also permit inhalant concentrations to be more readily adjusted, particularly lowered, during the exposure period. This can be accomplished by manually adjusting flowmeters to alter the proportion of inhalant laden and fresh air passing into the exposure chamber. A more sophisticated system might use a computerized gas blender composed of multiple mass flow sensors and proportional valves. The advanced capabilities of a dynamic exposure system are important advantages for some types of inhalant experiments but we have generally found them unnecessary for the brief exposures to single concentrations of inhalants that have been used in our drug discrimination studies to date.

3. Measurement of Inhalant Concentrations

As noted previously, one major advantage of a static vapor exposure apparatus is the ability to use the simple calculations of the ideal gas law to determine the amount of a volatile compound necessary to produce a given vapor concentration in the exposure chamber. The fact that the mathematical formula is based on a scientific law would lead one to believe that a calculated inhalant volume, when injected into the fixed volume of a static exposure chamber, will always yield the desired exposure concentration. In daily use we have found this assumption to be well founded, however, in practice it is advisable to verify static chamber inhalant concentrations on a regular basis for at least two reasons. The first is to insure that the exposure chamber is indeed a properly constructed, fairly well sealed enclosure. This is easily determined by measuring the degree to which the exposure chamber vapor concentration deteriorates over the anticipated exposure duration. A small loss of inhalant vapor is probably inevitable given that constructing a completely airtight chamber would be difficult. A large loss of inhalant vapor would obviously be more problematic. In our laboratory we have generally accepted a less than 10% decline from the desired inhalant concentration between the onset and the end of the exposure period. This degree of variance is likely comparable to the minor day-to-day inconsistencies encountered when injecting small volumes of test drugs in more traditional drug discrimination experiments.

The second reason for inhalant concentration monitoring is to determine the rate at which the inhalant under study is volatilized and distributed within the exposure chamber. The size of the exposure chamber, volume of liquid inhalant introduced,

efficiency of the internal fan and volatility of the inhalant being studied will all affect the rate at which a uniform exposure chamber vapor concentration is achieved. Due to the interplay of so many different variables, there is no general rule of thumb that can be used to abrogate the necessity of performing inhalant vapor concentration monitoring when a system is first constructed as well as when each new inhalant or inhalant concentration is examined. If an inhalant is volatilized slowly, either because a large volume of liquid is introduced, the internal fan is inefficient, or the inhalant is not particularly volatile, then the desired vapor exposure concentration might not be achieved until well into the exposure period or not at all. Take, for example, a hypothetical study in which one wished to expose animals to 6000 ppm of an inhalant vapor for 10 minutes. A problematic situation would occur if the rate of inhalant volatilization was such that it required 5 minutes of the 10 minute period before the vapor chamber reached a steady state concentration of 6000 ppm.

The primary method we have used for analyzing real time inhalant concentrations in our laboratory is by single wavelength infrared spectrophotometry. This technique quantifies the concentration of inhalant vapor in an exposure chamber atmosphere by assessing the degree of infrared absorbance produced by the inhalant. For many years we have used a Miran 1A infrared spectrophotometer coupled to either a paper chart recorder or more recently a computerized chart recorder system consisting of a laptop computer and Dataq Instruments data acquisition kit. In a static exposure system, a small vacuum pump is employed to draw a sample of the inhalant chamber atmosphere through a length of tubing attached to a threaded port in the exposure chamber. The inhalant vapor is then routed through the spectrophotometer detector cell and the vacuum pump before being returned by a second length of tubing to another port in the exposure chamber. This circuit forms a closed-loop maintaining the fixed volume of the static system. The percentage absorbance registered by the spectrophotometer is then compared to a calibration curve in order to convert the reading into a parts per million vapor concentration. In a dynamic system, the vacuum pump is unnecessary since the spectrophotometer can simply be inserted at some point within the gas flow path either at the vapor inlet or exhaust side of the exposure chamber, being careful not to restrict airflow.

Unfortunately, the Miran 1A is no longer manufactured but they are extremely simple and durable instruments, requiring little maintenance. Indeed the actual instruments used for some of the first inhalant behavioral studies published by Moser and Balster in 1981 [20] are still in use [21]. Fortunately, given their reliability, completely functional 1A models can still be obtained from various surplus instrumentation sources or even from Ebay. Other spectrophotometers may also be suitable but care should be taken in their selection. Many newer instruments are designed to measure vapor concentrations such as those that might be encountered at low levels in the workplace. The detector system of such an instrument might be overwhelmed when employed to measure the high concentrations of vapors used in inhalant drug discrimination experiments. In addition to an appropriate detection range, an instrument should also be sufficiently versatile to measure a number of different inhalants. Detection of different inhalant vapors is accomplished with the Miran 1A by selecting an IR emitter wavelength that provides optimal sensitivity to the vapor of interest. A number of less

expensive portable IR spectrometers currently marketed are designed with a single or at best a limited range of vapors in mind.

In the absence of an appropriate IR spectrophotometer an alternative, albeit considerably more cumbersome and technically demanding means of assessing inhalant concentrations in a vapor exposure apparatus is gas chromatography. In a static system, a small sample or even serial samples of the chamber atmosphere can be withdrawn via a needle septum which can be built into the chamber. The small size of these samples relative to the exposure chamber volume means that the measurement itself has no meaningful effect on the inhalant concentration within the exposure chamber. Virtually any gas chromatograph should provide acceptable sensitivity given the high concentrations of inhalants involved. Gas chromatographs can be fitted with a number of different detectors and the appropriate GC detector will be dependent upon the inhalant of interest. In our laboratory we have found that a flame ionization detector is very versatile since it is sensitive to most hydrocarbon compounds. We seldom use gas chromatography for routine exposure chamber monitoring since the IR spectrophotometer method is sufficiently accurate, less complex, and produces a real-time measurement. However, utilizing gas chromatography does have an advantage in that, in addition to verifying exposure chamber inhalant levels, it is can be used for the quantification of inhalant levels in blood and tissue [21–24].

4. Dosing Considerations with Inhalants

The route of administration of a test drug in a discrimination study could be chosen for a variety of reasons. Convenience, laboratory preference, or scientific necessity might all be a factor in this choice. Many drug discrimination studies use intraperitoneal (i.p.) injection or intragastric gavage (i.g.) because they are both a fairly simple means of repeated, daily drug administration in rodents. Subcutaneous (s.c.) dosing might also be used to circumvent the effects of first pass metabolism. In the absence of a compelling rationale, it might be pondered why one would choose to go to the trouble of volatilizing a compound and testing it as a vapor if it could simply be injected in its liquid state. Indeed many toxicology studies with inhalants dose by injection [25]. The response to this question is driven both by scientific and practical considerations. On the practical side, most abused volatile compounds are very poorly soluble or completely insoluble in aqueous vehicles at concentrations that produce acute pharmacological effects. This fact makes it necessary to use oil or fat emulsion vehicles to prepare appropriate injection concentrations. Unfortunately, even when highly diluted in a fat emulsion vehicle to the typical 10 ml/kg injection volume that our laboratory uses for i.p. dosing in mice, injections of solvent-based inhalants are noticeably aversive when given acutely. These concentrations may also produce harmful tissue irritation and inflammation if administered on the daily basis required for use as a discrimination training drug.

While practical considerations alone might not be sufficient to develop the necessary apparatus and expertise to deliver volatile chemicals via inhalation, there are also valid scientific reasons for doing so. The most obvious scientific rationale for examining volatile compounds by the inhalation route is because that is the means by which these

substances are abused. If one is only interested in utilizing drug discrimination to explore the neurochemical mechanisms underlying the effects of inhalants, this might or might not be a compelling rationale. Indeed, many drug discrimination experiments use dosing routes that don't mimic the route of abuse in humans and that has not diminished their importance. However, if in addition to understanding the neurochemical substrates responsible for the discriminative stimulus effects of inhalants one also wishes to assess the temporal aspects of that stimulus, then inhalation dosing is a necessity. Indeed, since the vast majority of abused inhalants cannot be examined in human laboratory studies due to their toxicity, exploring both the pharmacological substrates and temporal parameters of the discriminative stimulus of inhalants in animals is the only means by which such information will likely ever be systematically collected.

Regardless of the route by which a drug of interest is administered, the goal is to achieve behaviorally relevant drug concentrations in the blood and, by extension, the brain of the test subject. Drug discrimination training or test sessions are generally conducted using a drug pretreatment time estimated to correspond with peak plasma levels of a drug. In practice, however, the pharmacokinetic profiles of discrimination test drugs are rarely analytically quantified to determine optimal pretreatment times. Instead, pretreatment intervals are generally chosen based on those used in other behavioral studies reported in the literature. Evidence of substitution and/or operant rate suppression in subsequent drug discrimination test sessions is usually a sufficient means of confirming that appropriate doses and pretreatment times were selected. When examining injected or orally administered drugs in a discrimination assay this method, while perhaps not providing the most sensitive temporal measure of a drug's behavioral activity, is generally sufficient. The worst likely outcome is that somewhat higher doses of the test compound are necessary to produce substitution and/or response rate suppressing effects than would be the case if the drug was tested at an optimal pretreatment interval. When examining inhalants, some of which have extremely short durations of action following cessation of exposure [26, 27], a more thorough understanding of the kinetics of the compound being tested is often more critical in order to select optimal exposure and operant parameters.

In contrast to injected drugs, inhalants offer some unique dosing challenges beyond their limited duration of effect. Indeed, the entire concept of drug dose is more complex when dealing with inhalants, since it must also incorporate exposure duration as an important variable. Take, for instance, a situation in which an animal is being exposed to an inhalant in a static exposure chamber prior to drug discrimination testing. Due to the necessity of providing sufficient breathable oxygen, the exposure chamber is quite large relative to the subject. A chamber of this size usually requires that a fairly large volume of inhalant be introduced to produce behaviorally active atmospheric vapor concentrations. Under these conditions, absorption and metabolism of the inhalant vapor by the test subject will have a negligible effect on the overall exposure chamber vapor concentration. Inhalant blood concentration in the subject being exposed will, therefore, continue to rise until the exposure is discontinued or the concentration in the animal and the chamber atmosphere are in a state of equilibrium. The period of time required to reach equilibrium is dependent upon a great many pharmacokinetic factors and will differ between inhalants and between individuals. Some compounds, such as

TCE, may reach asymptotic blood and brain levels within a few minutes [21, 24]. In contrast, other compounds, like toluene, may require an extended period of time, perhaps as long as a few hours to reach equilibrium [28].

Fortunately, unless one is interested in discriminative stimulus effects resulting from environmental exposure, there is no particular necessity of exposing the subjects to an inhalant until their tissues are saturated. Indeed, for studies designed to examine the abuse-related discriminative stimulus effects of inhalants, long exposure durations that do not mimic the short duration intermittent human patterns of inhalant abuse are probably not scientifically appropriate. Rather it is considerably more practical and reflective of human exposures to simply choose a scientifically justified exposure duration and test a behaviorally active range of inhalant concentrations at that duration. In these cases, one can conveniently regard exposure chamber inhalant concentrations as analogous to injected drug dose. That being said, there are situations in which manipulating exposure duration can be experimentally useful. One of these is when an inhalant produces aversive peripheral effects, such as excessive lacrimation and salivation, at the concentrations necessary to elicit a behavioral effect during a short exposure. In these cases, extending the duration of inhalant exposure can, under certain circumstances, produce behavioral effects and inhalant blood levels that are functionally equivalent to a higher vapor exposure concentration over a shorter period of time [23].

5. Training and Testing Procedures for Use with Inhalants

Training and substitution testing methods for inhalants in drug discrimination procedures are generally similar to those using more traditional routes of administration, with a few notable caveats. In almost all of the inhalant discrimination studies published to date, the inhalant exposure and drug discrimination test sessions were conducted in separate apparati, necessitating the transfer of the test subject between the exposure and the test chamber. Since no inhalant vapor was present in the operant chamber, one must assume that inhalant blood levels declined over the course of the discrimination session. The degree to which inhalant blood, and presumably brain, levels fall during discrimination testing are a function of the inhalant under study, the length of inhalant exposure and the discrimination test session duration. Testing in the absence of continued exposure to the abused inhalant appears to have little effect following exposures to inhalants like toluene that have relatively high lipid solubility, leading to comparatively slow offset kinetics [23, 29]. In contrast, some inhalants, such as the newer volatile anesthetics like sevoflurane, were specifically designed to have extremely rapid onset and offset kinetics [26]. This fact can make capturing the discriminative stimulus and response rate suppressing effects of these compounds challenging. Figure 12-4 demonstrates this issue by showing the differences in the rates at which the discriminative stimulus effects of 10 minutes of exposure to toluene or TCE deteriorate following the cessation of exposure to their training concentration. Toluene substitution drops below the 80% threshold for full substitution after 10 minutes of room air exposure and still retains some partial substitution out to 30 minutes. In contrast, following cessation of exposure to the training concentration of TCE, substitution drops below 80% after only 3 minutes of room air exposure.

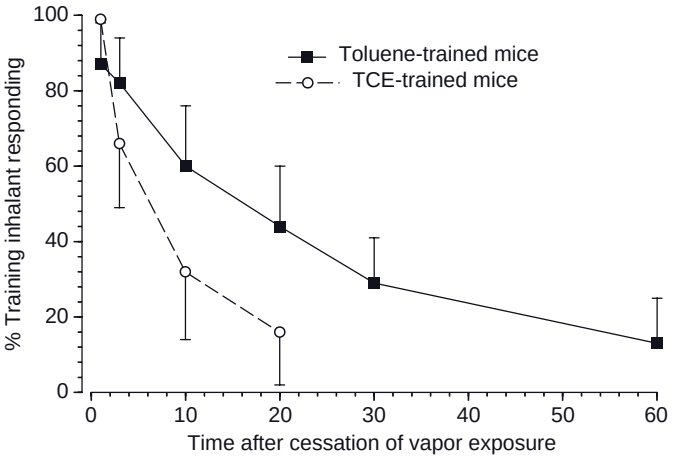

Figure 12-4. Rate of dissipation of the discriminative stimulus effects of toluene and TCE following the cessation of vapor exposure.

In studies in which animals have been trained to discriminate an injected drug such as pentobarbital or diazepam from vehicle, the methodological response to address the concern of inhalant effects dissipating during cross-substitution sessions has been to use fairly traditional 10–20 minute sessions with the training drug and short 2-minute cross-substitution test sessions with inhalants [30–32]. In more recent experiments in which we have trained inhalants themselves as discriminative stimuli, long training sessions would have led to an unacceptable decrease in internal inhalant concentrations over the course of the training session [21, 23, 33]. Instead we chose to decrease the duration of both the discrimination training and test sessions to 5 minutes. This was the shortest session length that we found maintained stable rates of reinforced responding across training sessions in mice. However, reducing the duration of the drug discrimination test session to 5 minutes or even 2 minutes is sometimes insufficient to capture transient response-rate suppressing effects of moderate concentrations of volatile inhalants or of high concentrations of inhalants with extremely short durations of action [29, 33]. Representative data showing the transient nature of the response-rate suppressing effects resulting from exposure to 10 minutes of 10,000 ppm isoflurane vapor is shown in Figure 12-5. The filled squares show mean operant response rates during consecutive 30-second segments across the entire 5-minute isoflurane test session. The open circles show similar data when the mice were exposed only to air for 10 minutes prior to discrimination testing. What is evident is that while operant responding is initially almost completely suppressed by 10,000 ppm isoflurane exposure (filled squares), the mice fully recovered within the first 2–3 minutes of the test session. In fact, response rates following isoflurane exposure actually exceed those following air exposure in the last 90 seconds of the test session. The reason for the delayed enhancement of response rates in the later portion of the test session after isoflurane exposure is unclear. The most plausible mechanism may be that the isoflurane-induced operant suppression early in the session prevented the animals from becoming satiated

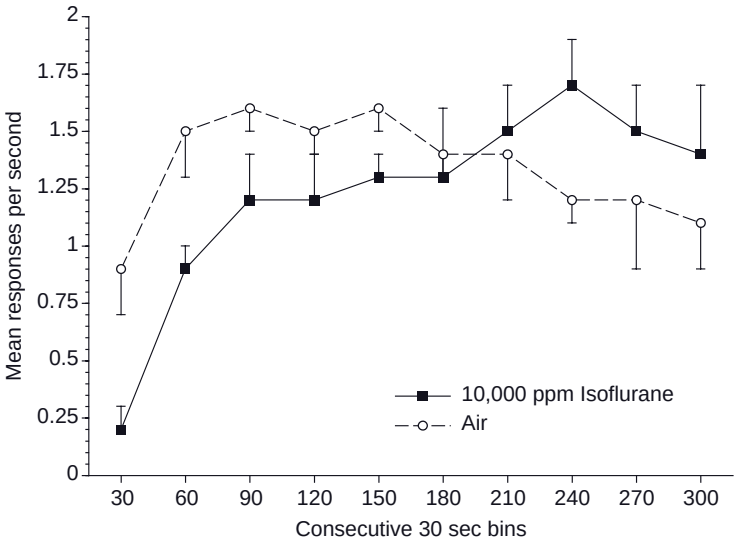

Figure 12-5. Mean response rates in responses/second for each of 10 30-second bins following exposure to air or 10,000 ppm isoflurane for 10 minutes.

with the sweetened milk reinforcer, as appears to occur in the air-exposed mice. Regardless, the net result of this pattern is that, when expressed as a mean 5-minute response rate, 10,000 ppm isoflurane appears to have minimal behavioral activity, which is clearly not the case. Based on this and similar data with other inhalants, we now routinely collect response rate data in 30-second bins during all discrimination test sessions to capture any transient response rate alterations produced by an inhalant.

C. NEUROTRANSMITTER SYSTEMS UNDERLYING INHALANT DISCRIMINATIVE STIMULUS EFFECTS

One of the most important uses of drug discrimination is for examining the neurochemical actions of drugs that may underlie their abuse-related behavioral effects. The first drug discrimination studies using inhalants preceded most of the in vitro experiments that have more fully elucidated the neurotransmitter systems affected by these compounds [34]. In order to appreciate the drug discrimination studies with inhalants that will be reviewed here, it will be helpful to briefly explore some of the neuropharmacology research on cellular mechanisms of actions of these agents.

There has been a small but steadily growing literature using electrophysiological techniques to examine the actions of abused inhalants on ion channel receptor function. These studies have shown that inhalants modulate ion flux mediated by endogenous neurotransmitters such as glutamate, GABA, serotonin (5-HT), glycine, and acetylcholine as well as effect voltage-gated calcium channels. For instance, toluene has been shown to attenuate NMDA-receptor mediated currents in recombinant NMDA receptors

but has no effect on non-NMDA glutamate channels [35]. In a subsequent study by the same author, a more extensive series of inhalants were examined [36]. The authors found that benzene, m-xylene, ethylbenzene, proplybenzene, and 1,1,1 trichloroethane (TCE) all selectively inhibited NR1/2B NMDA receptor activity at behaviorally relevant concentrations.

Abused inhalants also have effects on recombinant $GABA_A$ and strychnine-sensitive glycine receptors expressed in oocytes. Toluene, TCE, and trichloroethylene all enhanced GABA-mediated Cl^- flux in recombinant $GABA_A$ receptors as well as homomeric $\alpha 1$ glycine receptors [37]. The authors also demonstrated that TCE potentiated $GABA_A$ mediated IPSPs in rat hippocampal slices. Interestingly, rather than enhancing $GABA_A$ function, toluene was shown to inhibit $GABA_A$ receptor function in human receptors expressed in neuroblastoma cells [38].

Nicotinic acetylcholine receptors also appear be modulated by inhalants. Intraperitoneal injections of toluene at doses of 200 mg/kg or greater significantly decreased extracellular acetylcholine levels in rat striatum and hippocampus as measured by microdialysis [98]. Data from patch clamp studies in cultured hippocampal neurons, receptors expressed in oocytes, and human neuroblastoma cells found that toluene alone did not alter nicotinic receptor activity but significantly inhibited acetylcholine-induced currents [39, 40]. More recent experiments have extended these findings with toluene to perchloroethylene, showing that both rodent and human recombinant nicotinic acetylcholine receptors are inhibited by both inhalants, although perchloroethylene is significantly more potent than toluene in this respect [40]. It has also been shown that $5\text{-}HT_3$ receptor function is enhanced by inhalants. TCE, trichloroethylene, and toluene all dose-dependently potentiated 5-HT stimulated currents though $5\text{-}HT_3$ receptors expressed in oocytes [41].

In addition to ligand-gated ion channel receptors, inhalants can modulate the function of L-type voltage-dependent calcium channels. In pheochromocytoma cells, toluene application itself does not result in changes in intracellular calcium levels but does dose-dependently inhibit KCl^- -induced rise in calcium [42]. These results with toluene have been replicated and extended to perchloroethylene and TCE [43]. In the latter experiment, the rank order potency was also determined, showing that perchloroethylene was approximately 3 times more potent than toluene and 6 times more potent than TCE for inhibiting whole cell calcium currents.

As well as producing acute effects, prolonged exposure of cultured hippocampal neurons to toluene appears to selectively alter neurotransmitter receptor function, increasing sensitivity to NMDA and decreasing responsiveness to GABA stimulation [44]. Finally, exposure of animals to toluene produces increases in dopamine and 5-HT levels and decreases in dopamine turnover in brain areas associated with reward [45–49].

While these studies provide convincing evidence that abused inhalants can modulate neurotransmitter function, they do not address the question of whether these effects on isolated systems have any functional relevance to the behavioral effects and abuse liability of these compounds. It is in this area of study that the use of drug discrimination is uniquely suited. However, unlike most other drugs, in addition to CNS effects, inhalants have pronounced peripheral stimulus effects, most notably their strong odors.

It was recognized even in the earliest drug discrimination experiments with inhalants that potential interaction of CNS and peripheral stimulus effects would need to be addressed if studies examining neurotransmitter mechanisms were to be fruitful.

D. DISCRIMINATION CROSS-TEST STUDIES WITH INHALANTS

In considering the approach for conducting drug discrimination studies with inhalants, one is faced with the near impossibility of disassociating the strong odors of inhalants from their pharmacological effects and thus the difficulty of arranging for a placebo administration that retains the odors, but lacks the direct effects on brain and behavior. On the other hand, early studies of the direct effects of inhalation exposure to abused solvents on schedule-controlled behavior [18, 20, 50–52] were promising in that they provide evidence that the direct effects on the CNS were more responsible for these effects than were their odorant properties. For example, recovery of behavior occurred more slowly than the very rapid clearance of the odor from the chamber [18] and the potency differences between compounds were consistent with their potency differences for effects on motor performance [20, 53]. Nonetheless, several of the questions about inhalant intoxication could be answered by conducting cross-substitution studies in animals trained to discriminate various drugs from vehicle and then testing inhalants for substitution.

The first drug discrimination cross-substitution study with inhalants [34] was done using mice trained to discriminate 10 mg/kg of pentobarbital from saline using a two-lever operant procedure. Mice were tested following 20-minute exposures to various concentrations of toluene. Following the exposures, animals were moved within 60 seconds to the operant conditioning chamber and tested for only 2 minutes. Under these conditions, 8 of 10 mice fully generalized from pentobarbital to toluene. This occurred at concentrations of toluene that were just below those required to alter rates of responding. These results suggested that toluene produced pentobarbital-like subjective effects. It seemed very likely that direct effects on the brain were responsible for the substitution because it would be hard to imagine how toluene odors would be identified as pentobarbital-like. In a subsequent study in mice trained to discriminate 15 mg/kg pentobarbital from saline, TCE and the volatile anesthetic, halothane, produced high levels of partial, but not complete substitution [54]. In animals trained to discriminate an even higher, 20 mg/kg dose of pentobarbital from saline, TCE and halothane substituted even less robustly. The modest differences noted in cross-substitution profiles of inhalants for pentobarbital suggested that there may be subtle or perhaps less than subtle differences in the neurochemical effects produced by specific inhalants. This hypothesis was supported by a more recent study in mice trained to discriminate 2.5 mg/kg of the $GABA_A$ benzodiazepine-site positive modulator, diazepam, from saline. In that experiment, the volatile anesthetic methoxyflurane fully substituted for diazepam. In contrast, TCE only partially substituted and methoxyflurane completely failed to substitute for diazepam [55].

As previously noted, in vitro studies have indicated that the NMDA subtype of glutamate receptors are sensitive to the effects of several inhalants [35, 36]. In mice

trained to discriminate the uncompetitive NMDA receptor antagonist, phencyclidine (PCP), from saline, toluene produced high levels of partial substitution [55]. However, TCE, toluene, xylene, and methoxyflurane show little or no substitution in mice trained to discriminate a moderately high 1.7 mg/kg dose of the more selective and potent uncompetitive NMDA antagonist, dizocilpine ((+)MK-801), from saline [32].

Perhaps the most reproducible finding from inhalant cross-substitution studies thus far is that TCE, toluene, and isoparaffins, as well as a number of volatile anesthetics, show partial or full substitution in mice trained to discriminate ethanol from vehicle [29–31]. The discriminative stimulus effects of ethanol are likely mediated by actions on multiple neurotransmitters systems [56]. Thus far, it has been shown that $GABA_A$ positive modulators such as benzodiazepines and barbiturates substitute for ethanol [57–59]. NMDA receptor antagonists also fully or partially substitute for ethanol, dependent on the training drug and dose [59–65]. Lastly, serotonin $5\text{-}HT_{1B}$ agonists will also substitute for ethanol [66, 67].

The positive cross-substitution data with inhalants in mice trained to discriminate pentobarbital and diazepam and the data showing that barbiturates and benzodiazepines also substitute for ethanol suggests overlapping $GABA_A$ positive modulatory effects between ethanol and inhalants [34, 55]. In contrast, the inconsistent substitution of uncompetitive NMDA antagonists for inhalants [32, 55] as opposed to the fairly consistent finding that a number of NMDA antagonists substitute for ethanol would suggest some differences exist between the discriminative stimulus of ethanol and inhalants. One important finding from the ethanol literature that may have implications for the NMDA antagonist substitution data with inhalants is the observation that the ethanol training dose can alter the substitution pattern produced by other drugs. Low and intermediate ethanol training doses have been shown to have more prominent $GABA_A$ positive modulator-like [68] and $5\text{-}HT_{1B}$ agonist-like discriminative stimulus effects [66, 69]. Higher ethanol training doses, while still possessing GABAergic effects, also begin to produce uncompetitive NMDA antagonist-like discriminative stimulus effects [61]. It could therefore simply be the case that the doses of PCP and MK-801 trained in the prior inhalant cross-substitution studies were not behaviorally equivalent, leading to different outcomes when inhalants were tested.

Overall, cross-substitution studies with volatile inhalants suggest that the discriminative stimulus effects of inhalants may be mediated by multiple receptor systems [70, 71]. Positive $GABA_A$ receptor modulatory effects and NMDA antagonist effects are the two most probable mechanistic candidates, although other systems such as the serotonergic system should also be examined. The case for the GABAergic system is certainly the most compelling, but additional studies will need to be conducted with a number of different inhalants and site-selective NMDA antagonists to more clearly examine this possible mechanism. It appears that ethanol and inhalants interact through overlapping neurochemical systems to produce their discriminative stimulus effects. However, it may very well be the case that the relative contribution of individual receptor systems in transducing the discriminative stimulus effects of inhalants are not identical to ethanol and may very well differ to some degree across classes of inhalants.

A major difficulty in interpreting the literature on the substitution of inhalants in animals trained to discriminate a drug that has selective effects on a single receptor

type is the phenomena of asymmetrical substitution. Asymmetrical substitution occurs when drug A will substitute for drug B in animals trained to discriminate drug B, but drug B will not substitute for drug A in animals trained to discriminate drug A (see also Chapter 3). This pattern of results is classically seen with ethanol. Specifically, in animals trained to discriminate positive $GABA_A$ modulators or NMDA antagonists, ethanol tends to substitute poorly, if at all, whereas these classes of drugs substitute very well for ethanol [72–75]. This seeming paradox has been largely explained by data from a number of studies in which two dissimilar drugs were trained as discriminative stimuli both individually and as mixtures. As an example, in one experiment a mixture of pentobarbital and amphetamine was trained as a discriminative stimulus. The authors found that either drug alone would substitute for the mixture at sufficiently high doses but that the mixture would not substitute in rats trained to discriminate either pentobarbital or amphetamine alone [76]. Similar patterns of results in which the individual components of a drug mixture will substitute for the mixture have been shown with midazolam and nicotine [77] as well as with drugs from a number of other classes [[78] for review]. The general conclusion of these experiments is that the discriminative stimulus produced by components of a drug mixture are perceived separately from one another. As such, either component is sufficient to elicit drug mixture-appropriate responding. However, when an individual drug is trained as a discriminative stimulus and a mixture containing that drug and another drug is tested for substitution, the second drug, if in a sufficiently large dose, can overshadow the stimulus properties of the training drug in the mixture, resulting in no or only partial substitution (see Chapter 10).

Compounds that interact with multiple receptor systems, such as ethanol and potentially many inhalants, are analogous to drug mixtures. Therefore, when tested in animals trained to discriminate drugs selective for individual receptor systems, inhalants may fail to produce significant levels of substitution either because 1) there is no overlap between cellular mechanisms or 2) because of their mixed actions. Cross-substitution experiments cannot easily differentiate between these two outcomes; therefore, to fully elucidate the neurotransmitter systems responsible for the discriminative stimulus effects of inhalants they must be trained as discriminative stimuli.

E. INHALANTS AS TRAINING DRUGS

In the earliest studies of drugs as discriminative stimuli, there were concerns that peripheral stimulus effects such as local irritation resulting from injections or physiologically significant peripheral responses like increases in heart rate might underlie the discriminative stimulus [79–82]. Several experiments were conducted early in the evolution of drug discrimination to show that these peripheral drug effects did not overshadow the CNS effects of training drugs. As a result of these studies, as well as years of practical experience, there is no longer any real dispute that the discriminative stimulus effects of drugs are mediated by their central nervous system effects [83].

Unfortunately, unlike most drugs that probably have relatively modest peripheral stimulus effects relative to their CNS stimulus properties, the majority, if not all, abused volatile compounds have odor thresholds at concentrations that are orders of magnitude

below those that produce acute CNS effects. For instance, the odor threshold required for 100% accurate detection of the odor of toluene in man has been measured as 10 ppm or less [84]. In general, the subjective odors of the concentrations of inhalants that produce overt behavioral effects are quite intense. In addition to odor, high concentrations of some inhalants also produce lacrimation and salivation. In discrimination studies that employ inhalants as training stimuli, the salience of the inhalants peripheral olfactory stimulus effects could conceivably overshadow the compounds' CNS stimulus effects. Such an outcome would make drug discrimination experiments designed to assess the neurochemical systems underlying inhalants abuse-related subjective effects impossible.

The first study that was conducted with an inhalant as a training drug utilized 100 mg/kg i.p. injected toluene as a discriminative stimulus [85]. This route was chosen to minimize, but not eliminate, the possibility of the discrimination being based on the presence or absence of toluene odor. Animals trained to discriminate i.p. toluene generalized to inhaled toluene, with the concentrations producing full substitution being similar to those that produced pentobarbital-like effects in the Rees et al. study [34]. This generalization across routes of administration suggested that odor is not a major contributor to the discriminative stimulus effects of i.p. toluene. In this study, injected pentobarbital produced toluene-like effects, although only at high doses. This experiment also produced the first evidence for the selectivity of toluene-like discriminative stimulus effects, in that morphine produced only low levels of toluene-lever responding. A subsequent study [86] demonstrated that i.p. toluene could also be used for drug discrimination training in rats. In this experiment, methohexital and oxazepam substituted for toluene in most animals whereas chlorpromazine did not. This series of studies examining the CNS depressant drug-like discriminative stimulus effects of toluene, combined with other types of behavioral studies showing depressant-like drug effects (reviewed in [87]), led to the tentative conclusion that toluene, as well as some other solvents, were likely being abused because they produced a very rapid onset and offset depressant drug- or alcohol-like intoxication.

Although cross-substitution tests with inhalants continued to be conducted, there was a gap of 17 years before any subsequent drug discrimination studies were undertaken with abused inhalants as training drugs. At that time, we decided to abandon training inhalants by injection and instead sought to determine if they could be trained as discriminative stimuli using their normal, inhalation route of abuse. This decision was made with the knowledge that it would necessitate an entire series of control experiments to examine the importance of olfactory cues versus CNS effects as controlling variables over discriminative performance.

Unfortunately, it is a difficult task to demonstrate with absolute certainty that the peripheral effects of inhalants are not partially or totally responsible for their discriminative stimulus effects. A direct test of this hypothesis, such as chemically rendering test subjects anosmic and determining whether they can learn or maintain an inhalant discrimination, might seem a viable strategy. However, inhalants at concentrations that produce acute behavioral effects activate not only the olfactory system but also the trigeminal system. Unfortunately the trigeminal system is not affected by experimentally induced anosmia [84, 88]. Therefore, even if animals rendered anosmic retained

their ability to discriminate an inhalant, it would not serve as sufficient evidence to rule out peripheral stimulus effects as underlying the discrimination.

Since it is difficult or perhaps impossible to eliminate the peripheral stimuli associated with inhalant exposure, more indirect methods have been employed to address the basis of inhalants' discriminative stimuli. One tactic we have used in our laboratory to disentangle the olfactory versus CNS effects of inhalants is to manipulate inhalant exposure duration. In one experiment, 10 minutes of exposure to 6,000 ppm toluene vapor versus 10 minutes of exposure to air was trained as a discriminative stimulus in mice [33]. Real time IR spectrophotometry indicated that it required slightly less than 1 minute for our static exposure chambers to fully volatilize and distribute the volume of toluene required to produce a 6,000 ppm vapor concentration. We therefore hypothesized that 1 minute was sufficient to expose the mice to the same olfactory/trigeminal stimuli produced by our 10-minute, 6,000 ppm toluene vapor training condition. Drug discrimination substitution tests were then conducted with 6,000 ppm toluene exposure durations of 1, 3, 7, and 10 minutes. The results indicated that a brief exposure to 1 minute of toluene vapor engendered only air-appropriate responding. A minimum of 7 minutes of 6,000 ppm toluene exposure was necessary to produce full substitution for the 10-minute exposure training stimulus. These findings were systematically replicated in a second experiment in which the training stimuli were 10 minutes of exposure to 12,000 ppm TCE versus air [21]. Again, 1 minute of exposure to 12,000 ppm TCE vapor produced only air-appropriate responding. These findings suggested that a brief exposure to the olfactory/trigeminal stimuli associated with either toluene or TCE was insufficient to engender responding trained by a more prolonged, 10-minute exposure.

In a related experiment, rather than decreasing inhalant exposure duration, we instead examined whether an inhalant's discriminative stimulus concentration-effect curve could be shifted to the left by increasing the duration of vapor exposure [23]. We chose to examine toluene since it takes an extended period of exposure to produce steady state blood levels; thus, increasing exposure duration should also increase toluene blood concentrations [28]. Mice were again trained to discriminate 10 minutes of exposure to 6,000 ppm toluene vapor from air. After training, a 10-minute toluene exposure concentration-effect curve was determined. A second toluene concentration-effect curve was then conduced with a 20-minute exposure duration. We hypothesized that if peripheral stimulus effects were controlling responding then the 10- and 20-minute toluene exposure concentration-effect curves would be identical since increasing the exposure duration would not be expected to alter the peripheral effects of toluene. In contrast, we found that the 20-minute toluene exposure concentration-effect curve was shifted to the left. In addition, toluene blood concentrations, irrespective of exposure duration were predictive of toluene-lever selection.

The second line of evidence arguing that inhalant drug discriminations are based on CNS mechanisms is drawn from examining the conditions necessary to train an inhalant discrimination. In our prior study examining TCE vapor as a discriminative stimulus, it required a mean of only 27 sessions to train a 12,000 ppm TCE vapor versus air discrimination. However, despite in excess of 100 daily training sessions, a reliable discrimination could not be produced between 4,000 ppm TCE vapor and air [21]. High doses of injected training drugs have frequently been reported to be more readily trained

than low doses, an effect likely to be the consequence of the high dose producing more pronounced CNS effects [89]. The inability to train the 4,000 ppm TCE concentration as a discriminative stimulus is probably not surprising if it exerts only weak CNS effects. The question then becomes why were the olfactory/trigeminal effects of 4,000 ppm TCE vapor insufficient to serve as a discriminative stimulus. It is difficult to extrapolate odor thresholds from human studies to rodents, but the odor threshold for TCE in humans has been reported to be as low as 100–140 ppm [90, 91] and humans exposed to TCE vapor at a concentration of 350–450 ppm report symptoms such as dizziness, excitation, and eye irritation [92]. Mice have over 3 times the number of functional olfactory receptor genes compared to humans and are generally assumed to have equal or greater olfactory ability [93]. The low training concentration of 4,000 ppm TCE was far in excess of the odor threshold in humans suggesting the odor was likely detectable by the mice.

If one postulates that the odor of 4,000 ppm TCE vapor was detectable, it begs the question of why an olfactory discrimination was not established. Procedural differences between the optimal methods for training the CNS effects of drugs as discriminative stimuli compared to those used to train odors as discriminative stimuli could, in part, be responsible. Odor training procedures typically employ at least dozens, usually hundreds, of brief discrete odor trials per day that are presented in close temporal proximity to the choice response [94–96]. Under these conditions, very subtle odor discriminations can be engendered in mice [96]. In contrast, in our experiment we used a standard drug discrimination training procedure in which the mice were exposed to either air or TCE once daily. In addition, we first exposed the animals to an inhalant vapor in the exposure chambers and then removed them prior to testing in operant conditioning chambers. As such, the mice were exposed to vapors in a different environment from that in which the discrimination training sessions were conducted. This latter procedure, while effective as a means of training CNS-mediated discriminative stimulus effects of drugs, may not be the optimal procedure for training an odor as a discriminative stimulus. The present results appear to support this conclusion and provide a second line of evidence suggesting that the peripheral effects of inhalant vapors play, at best, a minor role in their discriminative stimulus effects under the common drug discrimination training conditions.

The finding that inhalants produce comparable substitution regardless of their route of administration provides a third line of circumstantial evidence that CNS mechanisms likely mediate their discriminative stimulus effects. Specifically, in addition to an early study showing that inhaled toluene vapor will substitute for an injected toluene training stimulus [85], we have more recently shown that injected liquid toluene produces full substitution in animals trained to discriminate 6,000 ppm toluene vapor from air [33]. Another related aromatic hydrocarbon inhalant, ethylbenzene, produced the same degree of partial substitution for 6,000 ppm toluene across administration routes. In a subsequent experiment, we also conducted toluene blood level analysis in the discrimination study in mice [23]. The initial finding that i.p. toluene substituted for inhaled toluene was replicated. In addition, the results indicated that toluene blood concentrations, regardless of whether they were achieved via inhalation or i.p. injection, were predictive of the level of substitution in discrimination test sessions.

One concern from our initial drug discrimination studies with inhaled drug discriminations with toluene and TCE was the finding that virtually all of the other inhalants tested in these studies produced at least partial substitution for the training stimulus. These results were probably not surprising given that all of the inhalants tested had CNS effects, and for those inhalants that had been evaluated to determine their cellular mechanisms, common neurotransmitter systems had been demonstrated [35–37]. However, an alternative explanation suggested at the time was that a detectable odor versus no odor discrimination had been trained. To address this hypothesis, we examined whether a compound with a strong odor, 2-butanol, would engender inhalant-appropriate responding in TCE vapor-trained mice [21]. Concentrations of 2-butanol up to 100 times that used in a prior olfactory discrimination study in mice [96] failed to produce any substitution for 12,000 ppm TCE. In addition, concurrent exposure of the mice to 30 ppm 2-butanol along with 12,000 ppm TCE had no effect on the ability of the training concentration of TCE to produce full substitution.

The final and perhaps the most convincing line of evidence that inhalant discriminations are based on CNS mechanisms are recent findings that some injected drugs will elicit substitution in inhalant-trained mice. We have recently collected data showing that select $GABA_A$ positive modulators will produce partial or full substitution in TCE-, toluene- or isoflurane-vapor trained mice (currently unpublished observations). An example of this data is shown in Figure 12-6.

In this experiment, mice were trained to discriminate 10 minutes of exposure to 6,000 ppm isoflurane vapor from air. In these animals, the $GABA_A$ receptor benzodiazepine site positive modulator, midazolam, dose-dependently substituted for isoflurane vapor. A maximum of 77% isoflurane-lever selection was produced at a dose

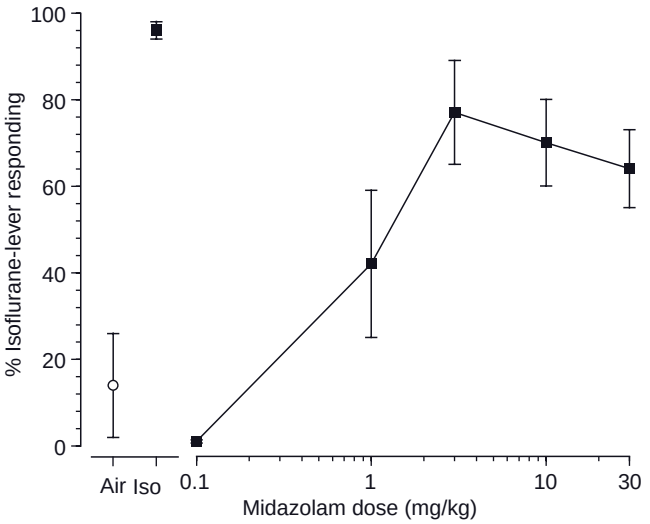

Figure 12-6. Substitution of i.p. injected midazolam in mice trained to discriminate 10 minutes of exposure to 6,000 ppm isoflurane from air.

of 3 mg/kg i.p. midazolam. Taken together with our previous studies, these data strongly support the hypothesis that inhalant vapor-based drug discrimination are indeed CNS-mediated. If one accepts this premise, then it can be concluded that inhalant discrimination procedures represent a viable means of studying the behavioral and neurochemical mechanisms underlying the discriminative stimulus effects of this class of abused drugs.

F. CONCLUSIONS

While to date the number of studies on the discriminative stimulus effects of inhalants are small, it is possible to reach some tentative conclusions relative to the initial set of questions posed at the outset of this chapter. The intoxications produced by toluene, TCE, and volatile anesthetics bear some similarity to that produced by benzodiazepines, barbiturates, and ethanol. These findings support the hypothesis that positive modulation of $GABA_A$ receptors plays a role in transducing the discriminative stimulus effects of at least some inhalants. The role of the NMDA receptor in the discriminative stimulus effects of inhalants is more uncertain and will require additional drug discrimination studies to unravel. Aside from one experiment indicating that toluene will substitute in amphetamine-trained mice [97], suggesting a dopaminergic component to toluene's discriminative stimulus, no other neurotransmitter systems have been systematically examined. The in vitro data suggest a number of potential candidates including the nicotinic acetylcholine, 5-HT, and opioid systems.

As to the question of whether inhalants all produce qualitatively similar intoxication, the data would suggest that there is substantial overlap between the discriminative stimulus effects of volatile hydrocarbon solvents and inhalant anesthetics. However, differences in substitution profiles between solvents and anesthetics as well as differences among representative members of the same class can be demonstrated [21, 23, 33, 55]. These data would, furthermore, suggest that there may also be some differences in the neurochemical actions among various solvents and anesthetics. Future studies in which representative inhalants are trained as discriminative stimuli and probed with compounds with known actions at specific neurotransmitter receptor sites will be required to address this question.

Finally there is the question as to whether inhalants differ in abuse liability and, if so, can drug discrimination be utilized as a scientific basis for recommending reformulation of abused products to lower their abuse liability. The combination of cross-substitution studies of inhalants in animals trained to discriminate known drugs of abuse along with the more recent development of procedures for training inhalants themselves as discriminative stimuli places this goal well within reach. Indeed the combination of these two drug discrimination procedures should allow the abuse-related subjective effects of inhalants to be examined in the same manner as is routinely done for novel pharmaceuticals with suspected abuse liabilities.

Acknowledgments The authors would like to acknowledge the editorial assistance of Katherine L. Nicholson, Ph.D., D.V.M., as well as support provided to Dr. Shelton by National Institutes on Drug Abuse Grant R01-DA017728.

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13

DRUG DISCRIMINATION STUDIES IN RHESUS MONKEYS: DRUG DEPENDENCE AND WITHDRAWAL

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A. INTRODUCTION

The popularity of drug discrimination procedures is evident by more than 4,000 publications in the Drug Discrimination Database (www.drugrefs.org), including more than 500 entries involving nonhuman primates. One strength of drug discrimination procedures is that they can be used in different species, including humans (see Chapter 3);

CPF is supported by DA05018 and DA17918 (Senior Scientist Award to CPF).

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

this chapter focuses on drug discrimination studies in nonhuman primates (rhesus monkeys) to illustrate the application of this methodology to studies of drug dependence and withdrawal.

The drug discrimination studies discussed in this chapter have been guided by theoretical concepts from classical receptor theory and by the following procedural guidelines: 1) determination of complete dose-response curves for drugs administered alone and for drugs administered in combination (agonists with antagonists and, although less common but not necessarily less important, agonists with agonists); 2) examination of drugs from multiple pharmacological classes; 3) examination of more than one drug from each pharmacological class; 4) characterization of drugs that are known to vary on the dimensions of affinity and efficacy; and 5) testing for generality of discriminative stimulus effects across different experimental conditions. By following these general guidelines the results of drug discrimination studies are amenable to analysis by quantitative pharmacological methods (e.g., Schild analysis).

B. SOME FACTORS IMPACTING THE DISCRIMINATIVE STIMULUS EFFECTS OF DRUGS

The particular conditions under which a drug discrimination procedure is established can dramatically impact the outcome. Conditions that are known to be important include the training drug and dose as well as the behavioral and drug histories of subjects, although other variables might also influence results of drug discrimination studies, including the reinforcer used to maintain responding and the schedule of reinforcement. Parametric studies on these and other conditions that are used to establish stimulus control can yield insights with regard to the mechanism of action of drugs as discriminative stimuli and they can help increase the efficiency of discrimination procedures. Following a brief overview of two-choice discrimination procedures, interactions between drugs administered acutely in these types of procedures, and some conditions under which stimulus control is established, this chapter discusses the application of discrimination procedures to the study of drugs under chronic dosing conditions in rhesus monkeys and how results from those studies might be related to drug dependence and withdrawal.

Drugs from a variety of pharmacological classes have been used as discrimination training stimuli in rhesus monkeys and a broad range of drugs has been tested in drug discrimination procedures (see Chapter 3). Drug discrimination procedures have high pharmacological selectivity; for drugs acting at receptors (e.g., μ opioid), typically only drugs acting in a similar manner (e.g., agonism) at the same receptor occasion responding on the lever associated with the training drug. Drugs that act at different receptors (or do not have adequate efficacy at the same receptor [see below]) do not occasion responding on the drug-appropriate lever (i.e., in a two-choice [drug versus vehicle] discrimination procedure, animals will press the vehicle [non-drug] lever). In rhesus monkeys, as in other species, pharmacological selectivity of the training stimulus is evident across many different training drugs. For example, in rhesus monkeys trained to discriminate the serotonin $(5\text{-HT})_{2A}$ receptor agonist 1-(2, 5-dimethoxy-4-

methylphenyl)-2-aminopropane (DOM) from saline, only drugs that have agonist activity at 5-HT_{2A} receptors occasion responding on the DOM-associated lever, including (+)lysergic acid diethylamide, (-)DOM, 2,5-dimethoxy-4-(n)-propylthiophenethylamine (2C-T-7), 2,5-dimethoxy-4-iodoamphetamine, dipropyltryptamine, and quipazine; drugs with antagonist actions at 5-HT_{2A} receptors and drugs acting at other receptors or other neurotransmitter systems do not share discriminative stimulus effects with DOM in monkeys [1].

The pharmacological selectivity of drug discrimination procedures can be influenced by the dose used for training. The relationship between training dose and pharmacological selectivity of a drug discrimination procedure has been studied in detail with rats [2]. In general, modifying the training dose can influence the apparent mechanism and selectivity of drug action. For example, decreasing the training dose decreases pharmacologic selectivity (i.e., increases the number and type of drugs that occasion drug-appropriate responding such that drugs differing in mechanism of action can produce drug-appropriate responding) and also decreases the efficacy requirements of the assay (i.e., decreases the amount of efficacy needed to occasion drug-appropriate responding). If the discriminative stimulus effects of a drug are mediated by multiple mechanisms, then increasing or decreasing the training dose can change the relative contribution of different mechanisms to the training stimulus, thereby altering the likelihood of particular drugs occasioning drug-appropriate responding. For example, the behavioral effects of ethanol are mediated by several different receptors, including gamma-aminobutyric acid $(GABA)_A$ and *N*-methyl-D-aspartate (NMDA) receptors. In rats discriminating a small training dose of ethanol (1 g/kg), the positive $GABA_A$ receptor modulator pentobarbital produces ≥80% ethanol-appropriate responding and the NMDA receptor antagonist dizocilpine does not; when a larger training dose is used (2 g/kg), dizocilpine produces ≥80% ethanol-appropriate responding and pentobarbital does not [3]. Thus, for a drug with multiple mechanisms of action, the contribution of different receptors can vary with training dose (see Chapter 3).

Even when the discriminative stimulus effects of a drug are mediated by a single population (type) of receptors, training dose can impact whether a drug occasions drug-appropriate responding because different training doses can be associated with different amounts of receptor activation. Consequently, drugs with less efficacy than the training drug are more likely to occasion drug-appropriate responding when a small training dose is used, as compared with a large training dose. When a lower-efficacy agonist fails to occasion responding on the drug-associated lever (e.g., under a large training dose condition), the lower-efficacy agonist should antagonize the effects of the higher-efficacy training drug. For example, nalbuphine produces ≥80% fentanyl-appropriate responding in rats discriminating 0.01 mg/kg of fentanyl from saline and not in rats discriminating 0.04 mg/kg of fentanyl from saline; nalbuphine attenuates the discriminative stimulus effects of fentanyl in the latter group [4].

Differences in efficacy requirements are most evident when different training doses are used; however, efficacy requirements can vary among subjects trained to discriminate the same dose of the same drug. In one study, four monkeys discriminating the positive $GABA_A$ receptor modulator midazolam responded predominantly on the drug-associated lever when tested with high-efficacy positive $GABA_A$ receptor modulators.

When the same monkeys were tested with the low-efficacy positive $GABA_A$ receptor modulator bretazenil, only three of the monkeys responded on the midazolam-associated lever. Studying drugs in combination in these monkeys provided evidence that a lack of responding on the midazolam-associated lever in the fourth monkey was due to differences in efficacy requirements among monkeys (and not, for example, to different receptors mediating these effects among monkeys). Bretazenil antagonized the discriminative stimulus effects of midazolam in the monkey that did not respond on the midazolam-appropriate lever after receiving bretazenil alone; the same doses of bretazenil enhanced the effects of midazolam in the three monkeys that responded on the midazolam-appropriate lever when tested with bretazenil alone [5]. Thus, bretazenil had agonist effects in three monkeys and antagonist effects in a fourth monkey, due to different efficacy requirements of the procedure among monkeys trained with the same dose of the same drug; this efficacy difference was not evident from studies with higher efficacy drugs which occasioned midazolam-appropriate responding in all four monkeys. Thus, the extent to which drugs with limited efficacy appear to be agonists or antagonists in drug discrimination studies depends, in part, on the efficacy requirements of the assay.

C. DRUG INTERACTIONS: ACUTE DOSING

As illustrated with the bretazenil study discussed above, drug interaction studies can be useful for identifying the mechanism(s) mediating discriminative stimulus effects and for characterizing properties (e.g., efficacy) of drugs and of assays. One of the simplest examples of a drug/drug interaction involves two drugs (i.e., an agonist and an antagonist) that reversibly bind to the same site (e.g., receptor). This type of antagonism often generates orderly data with the dose-response curve for agonist discriminative stimulus effects shifting to the right progressively and in a parallel fashion as the dose of antagonist increases. One way to express the magnitude of these shifts graphically is a Schild plot [6–10], for which ED_{50} values (the estimated dose needed to produce 50% responding on the drug-associated lever) are determined for an agonist administered alone and in combination with at least three doses of an antagonist. The dose ratios (agonist ED_{50} in the presence of the antagonist divided by agonist ED_{50} alone) are plotted as a function of antagonist dose (typically, -log molar dose). Data from three antagonist doses generate three data points that are fitted by linear regression, with two parameters being generated, slope and intercept. A slope that is not different from unity (−1) is consistent with the notion that the interaction between the agonist and antagonist occurs at a single site (receptor) and that the interaction is competitive and reversible. The intercept estimates the apparent affinity (pA_2) of an antagonist (i.e., estimated dose of antagonist to shift the agonist dose-response curve 2-fold rightward), which should not be different for an antagonist in combination with different agonists acting at the same receptor (i.e., so long as data are obtained in the same species with the antagonist administered by the same route).

Schild analysis is particularly useful for identifying or confirming the receptor(s) mediating the discriminative stimulus effects of an agonist. Agonists acting at the same

receptor often have qualitatively similar discriminative stimulus effects and those effects are blocked in a predictable manner (e.g., similar pA_2 values) by an antagonist acting at that receptor. For example, 5-HT_{2A} receptors appear to mediate the discriminative stimulus effects of DOM in rhesus monkeys as indicated by the ability of other drugs with agonist activity at 5-HT_{2A} receptors to occasion responding on the DOM-associated lever (e.g., 2C-T-7 and dipropyltryptamine) and by the ability of antagonists with affinity for 5-HT_{2A} receptors (e.g., MDL100907 [(*R*)-(+)-{α}-(2,3-dimethoxyphenyl)-1-[2-(4-fluorophenyl)ethyl]-4-pipidinemethanol]) to block the discriminative stimulus effects of those agonists [8]. The Schild plot for each agonist/MDL100907 combination yields regression lines that are not different from each other and not different from unity, suggesting that the interaction between the agonists and MDL100907 is competitive, reversible, and occurring at a single type of receptor. Apparent pA_2 values for MDL100907 in combination with DOM, 2C-T-7 or dipropyltryptamine are very similar (i.e., 8.50-8.61, corresponding to 0.9–1.2 μg/kg). The affinity of MDL100907 for 5-HT_{2A} receptors is more than 100-fold higher as compared with other (e.g., 5-HT_{1A} or 5-HT_{2C}) receptors; together with data from substitution studies, these results strongly suggest that the discriminative stimulus effects of DOM are mediated by 5-HT_{2A} receptors [8].

For other receptor systems, apparent affinity estimates for an antagonist are very similar across different conditions. For example, within a drug discrimination procedure, consistent pA_2 values are obtained for an antagonist in combination with a variety of agonists acting at the same receptor. In monkeys discriminating the positive $GABA_A$ receptor modulator midazolam, apparent affinity estimates for the neutral $GABA_A$ receptor modulator flumazenil in antagonizing triazolam and diazepam vary from 7.4 to 7.7 [11]. In monkeys discriminating the cannabinoid CB1 receptor agonist Δ^9-tetrahydrocannabinol (THC), affinity estimates for the CB1 receptor selective antagonist AM 251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl) pyrazole-3-carboxamide) in antagonizing the discriminative stimulus effects of THC, CP55940 (2-[(1*R*,2*R*,5*R*)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol), or WIN 55212-2 ((*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de)-1,4-benzoxazin-6-yl]-1-napthalenylmethanone) vary from 6.1 to 6.3 [12]. Moreover, similar pA_2 values for a particular antagonist are obtained in different drug discrimination procedures even when conditions are very different (e.g., untreated versus morphine-treated monkeys). The pA_2 value for naltrexone in antagonizing morphine is 8.21 in untreated monkeys and 8.19 in morphine-treated (i.e., dependent) monkeys [13]. The consistency of apparent affinity estimates across different agonists and different effects establishes the role of particular receptors in mediating the discriminative stimulus effects of drugs, confirms the pharmacological selectivity (i.e., for receptors) of each assay, and demonstrates the utility of Schild analysis for interpreting drug discrimination data within the framework of receptor theory.

Although Schild analysis is very useful for identifying mechanisms of action for discriminative stimuli, there are some limitations to its use. For example, this analysis requires the determination of full dose-response curves for the agonist alone and in the presence of at least three doses of antagonist, so these studies can be very labor intensive. With acute dosing, when one dose or dose combination is examined per session,

the completion of a full dose-response curve can require several weeks. Increasingly, these types of studies employ a cumulative dosing procedure whereby complete dose-response curves can be generated in one session. Though not necessarily a limitation, pharmacokinetic factors can dramatically impact pA_2 values; for example, one study determined pA_2 values for naltrexone in antagonizing the discriminative stimulus effects of morphine and varied the time between administration of naltrexone and determination of the morphine dose-response curve. As the time between antagonist and agonist administration increases, the apparent affinity (potency) of naltrexone decreased in an orderly fashion (from 8.43 to 7.08). However, across these conditions that generate significantly different pA_2 values, the nature of the interaction between naltrexone and morphine appears unchanged as indicated by slopes of the Schild plots that are not different across pretreatment times [14].

The interactions discussed thus far involve two drugs acting at the same site; however, two drugs can have qualitatively similar discriminative stimulus effects by acting at different sites on the same receptor complex and the effects of those drugs can be distinguished by the ability of site-specific antagonists to block the effects. For example, discriminative stimulus effects of positive $GABA_A$ receptor modulators are mediated by distinct sites on the $GABA_A$ receptor complex, which include benzodiazepine, barbiturate, or neuroactive steroid sites. Positive modulators acting at benzodiazepine and neuroactive steroid sites occasion drug-lever responding in monkeys trained to discriminate either a benzodiazepine or a neuroactive steroid; in both groups of monkeys the benzodiazepine site antagonist flumazenil blocks the discriminative stimulus effects of benzodiazepine site positive modulators (e.g., midazolam) and not those of neuroactive steroid site positive modulators (e.g., pregnanolone [Gerak, unpublished observation; 5, 11]). Thus, drug combination studies are useful beyond Schild analysis to examine similarities and differences among drugs and specific sites of drug action.

Although antagonists or drugs with limited efficacy (e.g., bretazenil [see above]) are most commonly combined with antagonists to study the mechanism by which agonists exert discriminative stimulus effects, agonists can also be studied in combination with other agonists. Interactions between two agonists that produce a full effect when administered alone should be additive, and when those agonists produce discriminative stimulus effects by acting at the *same* site, effects should not be different from combining different doses of the same drug. For example, in monkeys discriminating midazolam, other positive $GABA_A$ receptor modulators acting at the same (benzodiazepine) site occasion responding on the drug-associated lever (e.g., diazepam); in combination with midazolam, those drugs enhance its discriminative stimulus effects, shifting the dose-response curve leftward in an additive fashion [15]. The interaction between two agonists that produce qualitatively similar discriminative stimulus effects by acting at *different* sites can also be additive and, thus, indistinguishable from the interaction between drugs acting at the *same* site. For example, in monkeys discriminating midazolam, the neuroactive steroid pregnanolone occasions responding on the drug-associated lever; when administered in combination with midazolam, pregnanolone enhances its effects, shifting the dose-response curve leftward in an additive fashion [15].

When two agonists produce discriminative stimulus effects by actions on different sites and when those effects are qualitatively different, it can be difficult to predict the

effects obtained when the two drugs are administered in combination. However, this is an important area of research in light of the prevalence of polydrug abuse and the common practice of giving drugs in combination in the clinic. Stolerman and colleagues examined drug interactions with particular attention to drug combinations that are used as the discrimination training stimuli [16–18]; those studies showed orderly relationships between different training conditions and the results of substitution and antagonism studies in rats. In animals trained to discriminate a single drug from vehicle, unexpected results are sometimes obtained with combinations of drugs that *do not share* discriminative stimulus effects when studied alone. For example, cannabinoid and μ opioid receptor agonists do not share discriminative stimulus effects in rats [19] or monkeys [20] discriminating the cannabinoid receptor agonist THC. The discriminative stimulus effects of THC in rats are enhanced by the μ opioid receptor agonist heroin [19], whereas the discriminative stimulus effects of THC in monkeys are not altered by heroin [20]. Thus, the interaction between cannabinoid and μ opioid receptor agonists is qualitatively different between rats and monkeys. Even within the same species, drug interactions are not always symmetrical; for example, in monkeys the discriminative stimulus effects of THC are not altered by heroin whereas the discriminative stimulus effects of heroin are significantly attenuated by THC [20]. It is unclear why these drugs produce different effects across species and why the interaction between cannabinoid and μ opioid receptor agonists is asymmetrical in monkeys. However, it is clear that the discriminative stimulus effects of one drug can be significantly altered by drugs from distinct pharmacological classes and that these types of interactions have received comparatively little systematic investigation, despite their potential importance for understanding polydrug abuse and drug combination therapies.

D. DRUG INTERACTIONS: CHRONIC DOSING

Discrimination procedures also have been used to examine drug interactions when one drug is administered repeatedly (e.g., daily). Repeated agonist treatment can affect discriminative stimulus effects of drugs in several ways. First, repeated treatment can decrease the potency of that agonist (i.e., tolerance) as well as the potency of agonists with the same mechanism of action (i.e., cross tolerance). For example, daily treatment with morphine can produce tolerance to its discriminative stimulus effects (as well as the effects of pharmacologically equivalent agonists), as reflected by shifts rightward in the morphine dose-response curve [21]. Tolerance to discriminative stimulus effects can occur very rapidly, even after a single drug injection; for example, in monkeys discriminating midazolam, a single injection of chlordiazepoxide is sufficient to shift the midazolam dose-response curve rightward 24 hours later, indicating the development of acute tolerance [22].

In addition to decreased sensitivity to agonists (i.e., tolerance), repeated agonist administration can also increase sensitivity to antagonists, and this appears to be true for discriminative stimulus effects of antagonists as well. Stimulus control can be established and maintained with small doses of antagonists in agonist-treated subjects (so long as both agonist and antagonist act at the same receptor), although few

quantitative data are available regarding the potency of antagonists as discriminative stimuli between untreated and agonist-treated subjects [23, 24]. Early studies using antagonist discrimination procedures during chronic treatment with agonists were conducted with opioids in rats [25] and pigeons [23]. Subsequently, nonhuman primates were used for conceptually similar studies [26], and later the same approach was used to explore the discriminative stimulus effects of antagonists in monkeys treated chronically with a benzodiazepine [24] or a cannabinoid receptor agonist [27]. Monkeys are particularly well suited for these types of studies because, in the case of opioids, there is an extensive literature available on chronic treatment with a large number of opioid receptor agonists in monkeys (e.g., Drug Evaluation Committee of the College on Problems of Drug Dependence; www.cpdd.org). The relatively long life expectancy of monkeys in the laboratory also is an advantage because these procedures can require extensive periods of drug treatment and discrimination training.

Repeated treatment with an agonist can also produce physical dependence that is evident by the withdrawal that emerges following discontinuation of agonist treatment or administration of an antagonist. Under conditions that produce physical dependence, antagonists are readily discriminated from vehicle. For example, monkeys treated with μ opioid receptor agonists, in doses sufficient to produce physical dependence (as indicated with emergence of withdrawal signs when agonist treatment is temporarily discontinued), discriminate small doses of naltrexone from saline [28, 29]. However, the relationship between withdrawal and antagonist-appropriate responding is not always clear; remarkably similar results can be obtained across different chronic dosing conditions that vary markedly in the level of physical dependence generated. In fact, reliable stimulus control can be established with an antagonist in subjects receiving an agonist daily under conditions that do not generate clear signs of dependence (e.g., no discontinuation-induced withdrawal signs). For example, monkeys receiving only 3.2 mg/kg/day of morphine reliably discriminate naltrexone, although withdrawal signs are not evident when morphine treatment is discontinued [26].

A working hypothesis of the drug discrimination studies mentioned above is that daily agonist treatment produces dependence and that the discriminative stimulus effects of antagonists in agonist-treated monkeys are related to withdrawal. It is postulated that the basis of these discrimination procedures, and the quantitative readout of the assay, is that lever choice is directly related to the magnitude of withdrawal, and converging lines of evidence generally support this proposition, at least under some conditions. To the extent that an antagonist discriminative stimulus in agonist-treated subjects is related to withdrawal, it should be mimicked by discontinuation of agonist treatment, just as discontinuation of treatment or administration of an antagonist produces subjective reports of withdrawal in drug-dependent humans. There are several examples where discontinuation of agonist treatment results in a time-related increase in responding on the antagonist-associated lever (e.g., opioids [30]). If antagonist-appropriate responding (e.g., naltrexone [opioid receptor], flumazenil [benzodiazepine site on $GABA_A$ receptors], or SR 141716A [cannabinoid receptor]) is reflective of withdrawal, then it also should be reversed by re-administration of the dependence producing drug (e.g., morphine, diazepam, or THC, respectively) or its pharmacological equivalent, and that is generally the case. For example, the μ opioid receptor agonists

alfentanil, heroin, and morphine reverse responding on the naltrexone-associated lever that occurs when daily morphine treatment is discontinued [6, 26]. However, an alternate explanation to these findings is that the time-related emergence of responding on the antagonist-associated lever is due to the elimination of agonist; moreover, apparent reversal of antagonist-lever responding is due simply to the presence of agonist. That is, the antagonist discriminative stimulus in agonist-treated monkeys might reflect the presence (vehicle-appropriate responding) or absence (antagonist-appropriate responding) of agonist effect.

This situation is not easily resolved by results of substitution studies or by results obtained after discontinuation of agonist treatment. However, information regarding the relationship between discriminative stimulus effects and withdrawal can be obtained from studies that concurrently measure discrimination performance and other behavioral or physiological effects that reflect the emergence of withdrawal. For example, in morphine-treated rats discriminating naltrexone, responding on the naltrexone-appropriate lever is correlated with weight loss and weight loss is one common indicator of opioid withdrawal in rats [31]. Temporary discontinuation of morphine treatment (5.6 mg/kg/12 hr) in rhesus monkeys results in the emergence of naltrexone-appropriate responding which covaries with directly observable and physiological withdrawal signs, including increased heart rate and activity; increased activity is particularly evident during the dark period, suggesting that opioid withdrawal disrupts sleep patterns [29]. Similarly, in monkeys treated chronically with diazepam and discriminating flumazenil, there is a strong positive correlation between responding on the flumazenil-associated lever, decreases in operant behavior (often used to measure drug withdrawal [e.g., 31]), in some cases seizure-like behavior, and the elimination of diazepam and its metabolites from blood [32]. Just as subjective reports (symptoms) of withdrawal in humans often diminish within days of discontinuing drug treatment, rhesus monkeys switch responding, from the antagonist-appropriate lever to the vehicle-appropriate lever, within several days or a week after discontinuation of chronic drug treatment. While discriminative stimulus effects of withdrawal are no longer evident a week after discontinuation of morphine treatment, other indices of withdrawal persist for much longer [29]. Thus, when sufficiently large doses of agonist are administered chronically in drug discrimination studies, antagonist-appropriate responding is accompanied by other withdrawal signs; such results support the notion that antagonist discriminations in agonist-treated monkeys are, at least under some conditions, reliable indices of withdrawal.

Many of the important features of drug discrimination procedures that render them especially useful for studying specific mechanisms of agonist action are maintained in antagonist discriminations in agonist-treated subjects. For example, antagonist discrimination procedures are pharmacologically selective. In agonist-treated monkeys discriminating an antagonist, other antagonists from the same pharmacological class occasion antagonist-appropriate responding. Whereas the potency of agonists as discriminative stimuli is related to (predicted by) their efficacy and affinity, the potency of antagonists is related only to their affinity. Thus, given similar bioavailability, the rank order potency of antagonists in producing discriminative stimulus effects in agonist-treated subjects is highly correlated with their receptor binding affinities [6, 33]. Moreover, and as predicted by receptor theory, discriminative stimulus effects of

antagonists can be blocked by an additional acute injection of the treatment agonist or a pharmacological equivalent. For example, in morphine-treated monkeys discriminating naltrexone, the opioid receptor antagonists naloxone, nalorphine, and quadazocine increase naltrexone-appropriate responding and an additional injection of morphine before the session shifts the antagonist dose-response curves rightward [26, 34].

The effects of drugs on operant responding can vary among different reinforcers, even when the ongoing rate and pattern of responding is indistinguishable across those conditions [35]. One possibility is that the discriminative stimulus effects of drugs also vary among procedures using different reinforcers. Discrimination procedures using agonists as training drugs often use food to maintain responding, but schedules of food presentation might not be appropriate for antagonist discrimination procedures in agonist-treated subjects. Antagonist discrimination procedures in morphine-treated pigeons used access to mixed grain to maintain key pecking [23, 36]. However, one sign of opioid withdrawal in humans is loss of appetite [37], and opioid dependent monkeys sometimes refuse food after receiving an opioid receptor antagonist. Moreover, responding maintained by a schedule of food presentation is sometimes disrupted by smaller doses of drugs as compared with responding maintained by schedules of stimulus-shock termination (unpublished observation, [38]). Thus, it appeared prudent to use a non-appetitive reinforcer when training morphine-treated monkeys to discriminate an opioid antagonist so as to reduce the possibility of monkeys not responding when they receive an antagonist, presumably because of withdrawal-related effects on appetite. Consequently, antagonist discriminations in morphine-treated rats [31], morphine-treated monkeys [26], and THC-treated monkeys [27] almost exclusively use schedules of shock escape/avoidance, which maintain stable responding in agonist-treated subjects even after administration of an antagonist [26]. For most discrimination procedures it is not clear whether similar results could be obtained with other reinforcers (e.g., food). However, when the same antagonist discrimination was trained in separate groups of agonist-treated monkeys (flumazenil in diazepam-treated monkeys), one group responding under a schedule of food presentation and a second group responding under a schedule of stimulus-shock termination, there was no difference in discriminative stimulus effects of a variety of drugs [24]. Although few data are available on this topic, it appears as though the particular reinforcer used in a drug discrimination study does not affect the qualitative features of the discriminative stimulus although it might impact the susceptibility of responding to disruption, thereby potentially limiting the dose ranges that can be studied.

Another important feature for antagonist discrimination procedures in agonist-treated subjects is the pharmacokinetic profile of the treatment drug. For example, the first studies using flumazenil as a discriminative stimulus in drug-treated monkeys used the very long acting benzodiazepine chlordiazepoxide [39]. While treatment with a long-acting agonist has the advantage that drug can be administered relatively infrequently, there is a potential disadvantage to using long-acting agonists. When chlordiazepoxide treatment is discontinued in monkeys discriminating flumazenil, monkeys continue to respond on the vehicle-associated lever (i.e., not on the flumazenil-associated lever) for at least 9 days and no directly observable withdrawal signs are evident. The very slow elimination of chlordiazepoxide might cause a very gradual change in physi-

ology that is not qualitatively similar to the rapid change(s) that occurs after administration of an antagonist. Thus, the slow elimination of drug after discontinuation of agonist treatment does not appear to mimic the discriminative stimulus effects of antagonists that likely are associated with very rapid changes in physiology (i.e., receptor stimulation). In other studies, monkeys received a different benzodiazepine, diazepam, which has a comparatively shorter duration of action [24, 32, 40, 41]. The flumazenil discriminative stimulus in diazepam-treated monkeys appears to be qualitatively similar to the flumazenil discriminative stimulus in chlordiazepoxide-treated monkeys, and discontinuation of treatment with the shorter-acting diazepam results in some responding on the flumazenil-associated lever; however, even this effect occurs over several days and it is not temporally consistent among monkeys (e.g., perhaps related to individual differences in the metabolism and elimination of diazepam and its active metabolites). Substituting a benzodiazepine with a still shorter duration of action than diazepam (i.e., lorazepam; unpublished observation, [32]) does not appear to impact stimulus control with flumazenil; however, all monkeys respond on the flumazenil-associated lever within 24 hours of the temporary discontinuation of lorazepam treatment. Thus, the duration of action of the agonist administered chronically determines the rate of drug elimination and, therefore, possibly the qualitative aspects of the withdrawal (e.g., discriminative stimulus effects) that emerges upon discontinuation of treatment.

E. SUMMARY AND CONCLUSION

One advantage of drug discrimination procedures is that they generate qualitatively similar data across different conditions (e.g., species); moreover, the factors that contribute to the selectivity and sensitivity of discrimination procedures are well described and include the drug(s) and dose(s) used for training. These procedures have been used productively for many different areas of investigation, including studies on drug/drug interactions. Receptor theory provides a framework for designing and interpreting experiments and, despite the complexities that are inherent to behavioral studies on drugs (e.g., pharmacokinetic factors), results of drug discrimination studies are amenable to rigorous analysis by classical pharmacological methods, including Schild analysis. Discrimination procedures using an antagonist as the training stimulus in subjects that are treated chronically with an agonist can be used to study drug dependence and withdrawal for several different classes of drugs and, at least under some conditions, discriminative stimulus effects appear to be specifically related to withdrawal. Whether drug discrimination procedures can be advanced further to explore the qualitative features of dependence and withdrawal is yet to be determined, although progress in this area could provide a new and fertile application for this behavioral methodology.

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14

HUMAN DRUG DISCRIMINATION: METHODOLOGICAL CONSIDERATIONS AND APPLICATION TO ELUCIDATING THE NEUROPHARMACOLOGY OF AMPHETAMINES

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

A. INTRODUCTION

Drugs of abuse produce internal stimulus effects that can control behavior much like external stimuli. These internal effects and the ensuing stimulus control have been widely studied in nonhuman laboratory animals using drug discrimination procedures. In a typical drug discrimination experiment, a behavior (e.g., lever pressing) is differentially reinforced contingent on the presence or absence of a specific drug stimulus. For example, following the administration of the training dose (e.g., 0.056 mg/kg methamphetamine), responding on the left lever is reinforced (e.g., food pellet delivered). Following the administration of vehicle or saline, responding on the right lever is reinforced. Under this arrangement, drugs from diverse pharmacological classes have been shown to exert discriminative control of behavior in different species [1; see also Chapters 2 and 3]. The drug discrimination procedure has at least three notable strengths. Drug discrimination is pharmacologically sensitive in that larger doses of the training drug generally engender increased drug-appropriate responding. Across a sufficient range of doses the dose-response curve for the training drug is quite steep, with at least one dose producing minimal effect while at least one dose produces near maximal drug-appropriate responding. Drug discrimination is also pharmacologically selective in that drugs from the same class as the training drug generally increase drug-appropriate responding as a function of dose, while drugs from different classes generally produce placebo or not drug responding [1]. The results of drug discrimination studies also correlate well with drug actions at the cellular level [2].

Drug discrimination procedures have been adapted for use with humans. Below, the extant literature that assessed the discriminative-stimulus effects of drugs in humans is reviewed. Since the adaptation of drug discrimination procedures for use with humans, a number of reviews have been published. These reviews focused on: a) the relationship between the discriminative-stimulus and subjective effects of drugs [3–5]; b) the concordance between preclinical and human drug discrimination experiments [6]; and c) the neuropharmacological selectivity of drug discrimination procedures relative to subjective drug-effect questionnaires [7]. The present chapter differs from these

previous reviews in that it focuses on methodological issues that must be considered when designing and conducting a human drug discrimination study. This chapter is not intended to review every available study that used human drug discrimination procedures. Instead, when possible, studies that used an amphetamine to assess the discriminative-stimulus effects of drugs in humans are reviewed for illustrative purposes. The present chapter then discusses the utility of human drug discrimination procedures to elucidate the neuropharmacological actions of amphetamines.

B. METHODOLOGICAL ISSUES TO CONSIDER WHEN DESIGNING AND CONDUCTING A HUMAN DRUG DISCRIMINATION EXPERIMENT

There are over 100 published studies that used drug discrimination procedures to assess the behavioral effects of drugs in humans (Figure 14-1). Not surprisingly, the experimental methods used in these experiments varied considerably. Here, issues relevant to the design and conduct of a human drug discrimination experiment are discussed. This section is not intended to be exhaustive. Rather, the intention is to highlight some of the methodological issues that must be considered when conducting drug discrimination experiments with human participants.

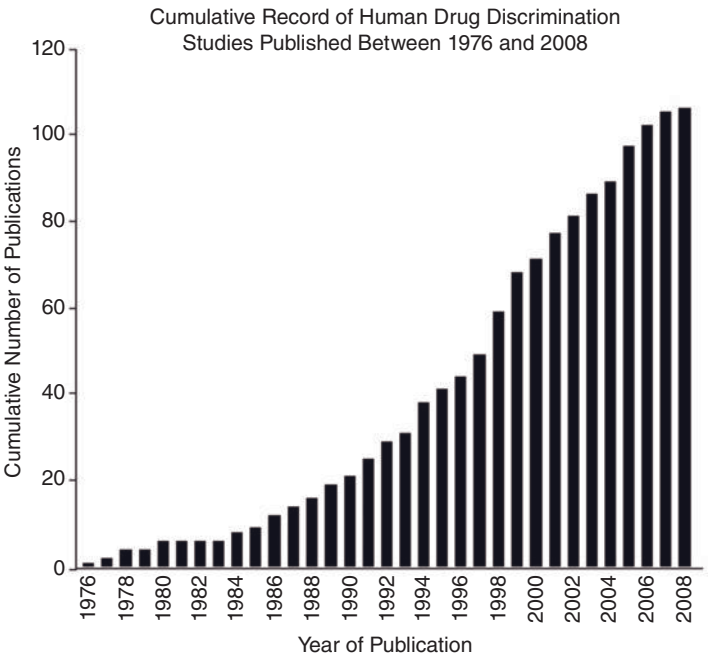

Figure 14-1. Cumulative number of human drug discrimination studies published between 1976 and 2008.

1. General Methodological Considerations

The methods used in human drug discrimination studies are very similar to those used in preclinical experiments. Human drug discrimination experiments often consist of three phases that are completed in fixed order: 1) Sampling Phase; 2) Acquisition Phase; and 3) Test Phase. During the sampling phase participants complete experimental sessions to acquaint them with the effects of the training dose. The training dose is usually identified to participants using a code (e.g., Drug A or Red Drug). Participants may also complete sampling sessions during which they receive placebo. In this case, placebo is identified with a unique code (e.g., Not Drug A, Drug B, or Blue Drug). During the sampling sessions participants are verbally instructed to pay attention to the drug effects because in future sessions correctly identifying the drug they received will be reinforced. The reinforcer in human drug discrimination experiments is almost always money. Participants complete subjective drug-effect questionnaires periodically for several hours after drug administration during the sampling phase. Following the sampling sessions, an acquisition phase is conducted. The training dose and placebo are administered several times in random order during the acquisition phase. Volunteers ingest drug or placebo during each acquisition session, but the code is not revealed to the participant until the end of the session. During this phase, participants complete the drug discrimination task along with subjective drug-effect questionnaires periodically for several hours after drug administration. At the end of each acquisition session, participants are informed of the drug condition they received that session (i.e., Drug A or Not Drug A, Drug A or Drug B, Red Drug or Blue Drug). The percent of correct responding is converted to money and the participant is told immediately how much bonus money he/she earned during the experimental session. The criterion for having acquired the discrimination is predetermined (e.g., 80% correct responding on four consecutive days), and only those participants that meet the criterion in a specified number of sessions (e.g., 12) advance to the test phase. The extensive training that is included as part of the human drug discrimination procedure provides participants with similar recent behavioral and pharmacological histories. Extensive training may reduce variability both within and across participants.

During the test phase the discriminative-stimulus effects of different doses, drugs, or drug combinations are determined. Sessions involving the administration of doses or drugs other than the training condition are deemed to be “test” days. Participants are not told the purpose of “test” sessions, nor do they know when these sessions are scheduled until completing the session. There is no correct responding *per se* on these “test” sessions, so participants usually receive all of the available money that is contingent on correctly identifying the drug condition that was administered. Acquisition sessions are interspersed among “test” sessions to ensure that participants continue to accurately discriminate the training condition. Additional sessions are conducted to re-establish accurate discrimination if the participant fails to correctly identify the training condition they received on an acquisition session conducted during the test phase. The number of acquisition sessions included in the test phases varies but is usually a percentage of the total number of test sessions (e.g., 25–50%).

In general, there are two permutations of the human drug discrimination paradigm: 1) substitution of other drugs, and 2) pretreating participants with compounds (i.e., antagonists) that might modify the discriminative-stimulus effects of the training drug. In all experiments, participants first learn to discriminate a drug (e.g., d-amphetamine). After acquiring the discrimination in a substitution experiment, a dose-response curve is determined for the training drug. A range of doses of other drugs (e.g., methylphenidate or bupropion) is then tested to determine whether they share discriminative-stimulus effects with the training drug. Based on the drugs that engender significant drug-appropriate responding, inferences can be made regarding the neuropharmacological mechanisms that mediate the effects of the training drug. If, for example, a novel drug substitutes for d-amphetamine, the inference might be that the novel drug acts via central dopamine systems.

In pretreatment experiments, after acquiring the discrimination, a dose-response curve is established for the training drug (e.g., d-amphetamine). The dose-response curve for the training drug is then re-determined following pretreatment with pharmacologically selective antagonists. Inferences are made regarding the neuropharmacological mechanisms that mediate the discriminative-stimulus effects of the training drug based on the antagonists that shift the dose-response curve rightward.

2. Drug Discrimination and Subjective Drug Effect Questionnaires

Perhaps the most obvious consideration is whether the use of human drug discrimination procedures is appropriate for addressing the experimental question (e.g., elucidating the neuropharmacology of drugs or determining the influence of instruction). The internal stimulus effects of drugs are most often assessed in humans using subjective drug-effect questionnaires. The premise of these studies is that the subjective effects of abused drugs contribute to their abuse. Some of the questionnaires are standardized (e.g., Addiction Research Center Inventory [ARCI]), while others are investigator developed (e.g., Drug-Effect Questionnaire). In experiments that use these instruments, participants receive a range of doses of different drugs (e.g., d-amphetamine and methylphenidate) and then periodically complete a battery of subjective drug-effect questionnaires. Inferences concerning neuropharmacological and behavioral similarities or differences between drugs can then be made based on the constellation of subjective drug effects reported by participants. Drugs that produce a similar constellation of subjective effects, for example, would be predicted to share a common mechanism of action. Alternatively, participants are administered a range of doses of an abused drug (e.g., d-amphetamine) alone and following pretreatment with another drug (e.g., a dopamine receptor antagonist). Inferences regarding the neuropharmacological mechanism that mediates the effects of the abused drug can then be made depending on the pretreatment drugs that attenuate the subjective drug effects.There is considerable evidence to suggest the discriminative-stimulus and subjective effects of drugs overlap extensively. The relationship between the discriminative-stimulus and subjective effects of drugs has been comprehensively reviewed previously [3–5]. Thus, for illustrative proposes we describe the results of a previous study conducted in our laboratory that demonstrates the extensive overlap

between the discriminative-stimulus and subjective effects of drugs [8]. In this study, participants (N = 7) learned to discriminate 10 mg oral methamphetamine. After acquiring the discrimination (≥80% drug-appropriate responding on four consecutive sessions), a range of oral doses of methamphetamine (2.5–15 mg), d-amphetamine (2.5–15 mg), methylphenidate (5–30 mg), and triazolam (0.0625–0.375 mg) were tested. d-Amphetamine and methylphenidate were tested because they are structurally similar to methamphetamine. Triazolam, a triazolobenzodiazepine hypnotic that exerts its effects at the benzodiazepine recognition site of the γ-aminobutyric acid-A ($GABA_A$) receptor complex, was included as a negative control [9]. Methamphetamine functioned as a discriminative-stimulus and produced prototypical stimulant-like subjective effects. d-Amphetamine and methylphenidate increased drug-appropriate responding as a function of dose, whereas triazolam did not (Figure 14-2). d-Amphetamine and methylphenidate produced stimulant-like subjective effects, while triazolam produced sedative-like effects. There were no discernible differences between methamphetamine, d-amphetamine, and methylphenidate in terms of their subjective effects profile.

While the results of the study described above suggest extensive overlap, it is important to emphasize that the discriminative-stimulus and subjective effects of drugs are not isomorphic [10–13]. The combined use of a drug discrimination procedure and subjective drug-effect questionnaires may, therefore, more fully characterize the behavioral effects of drugs. In a previous study conducted in our laboratory, six participants with recent histories of nontherapeutic stimulant use learned to discriminate 30 mg oral methylphenidate [11]. After acquiring the methylphenidate discrimination, a range of doses of methylphenidate (5–30 mg), atomoxetine (15–90 mg), d-amphetamine (2.5–15 mg), triazolam (0.06–0.375 mg), and placebo were tested. A battery of subjective drug-effect questionnaires was also included to more fully characterize the behavioral effects of the drugs. At least one dose of methylphenidate and d-amphetamine increased drug-appropriate responding significantly above placebo levels, while none of the atomoxetine doses tested did so. Interestingly, the two highest doses of methylphenidate, d-amphetamine, and atomoxetine increased scores on a stimulant-sensitive adjective rating scale significantly above placebo levels. These findings are concordant with previous findings that found discordance between the discriminative-stimulus and subject-rated effects of drugs [10, 12, 13].

3. Effects of Route of Administration on Central and Peripheral Drug Effects

The route by which drugs are administered is another factor that must be considered when designing a human drug discrimination experiment. The ecological and external validity of the study are increased if a route used in the natural environment is utilized to administer the experimental medications. Some commonly abused drugs, however, produce robust peripheral effects when administered by routes commonly used in the natural environment. Interpreting the results of these experiments is difficult when the discriminative-stimulus effects of the drug are possibly mediated *via* peripheral rather than central mechanisms.

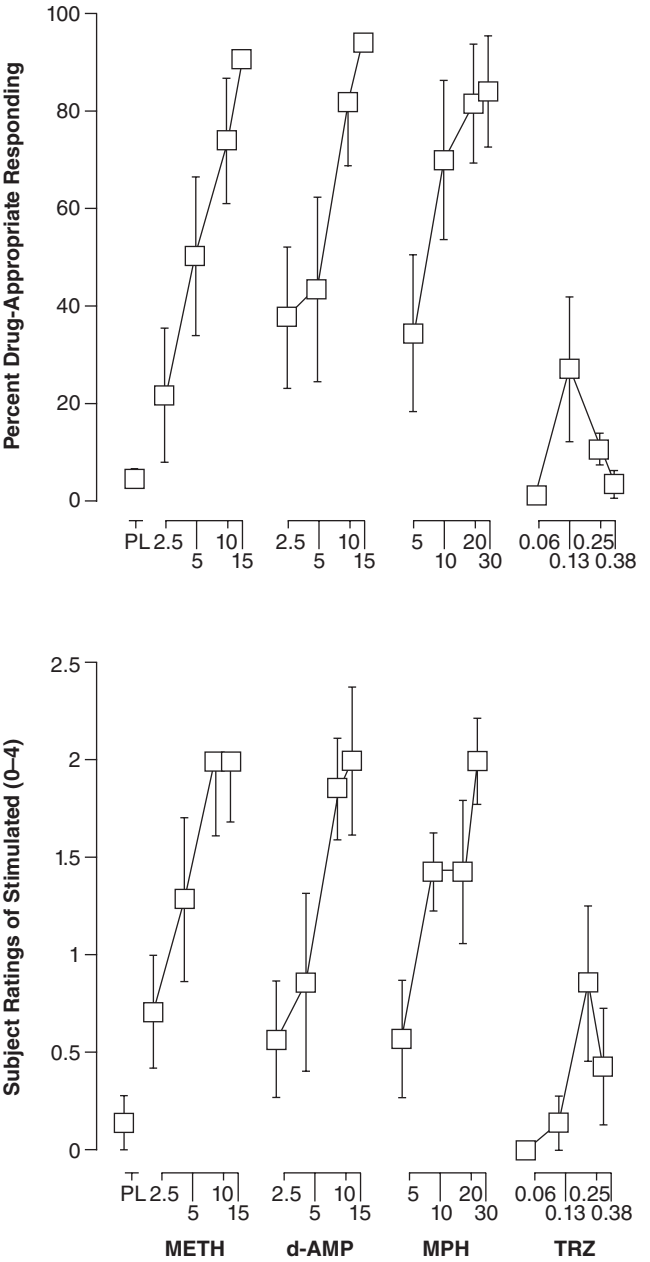

Figure 14-2. Dose effects for methamphetamine (METH), d-amphetamine (d-AMP), methylphenidate (MPH), and triazolam (TRZ) for percent drug-appropriate responding on a point-distribution drug-discrimination task along with subject ratings of Stimulated from a Drug-Effect Questionnaire. X-axes: dose in mg. Data points above "PL" designate values from the placebo "test" session. Data points show means of seven participants; brackets show 1 S.E.M. Redrawn from Sevak et al. (2009).

Cocaine, for example, is insufflated (i.e., snorted) in the natural environment. In addition to central effects, intranasal cocaine produces local anesthetic effects (i.e., numbing of the nasal mucosa). In one study, participants (N = 3) learned to discriminate between intranasal cocaine (50 mg) and placebo (46 mg lactose plus 4 mg cocaine) [14]. Low cocaine doses (e.g., 4 mg) produce nasal numbing but no discernible blood levels and are routinely used as the placebo dose in intranasal cocaine studies with humans [15–17]. The investigators attempted to further mask the dose conditions by applying benzocaine (20%) to the nasal mucosa. These participants reliably discriminated between the dose conditions approximately 15 seconds after drug administration. Because of the ultra-rapid onset of the discriminative-stimulus effects of intranasal cocaine, the authors concluded that the volunteers were likely basing their discrimination on peripheral rather than central cues. The results of this study demonstrate the potential contribution of peripheral cues in mediating the discriminative-stimulus effects of cocaine. Peripheral cues may also contribute to the discriminative-stimulus effects of oral alcohol and smoked marijuana [18, 19].

Human drug discrimination procedures, as discussed below, are sometimes used to determine the neuropharmacology of abused stimulants. The peripheral drug cues associated with some routes of administration used in a natural environment are especially problematic when utilizing the human drug discrimination paradigm for this purpose. The training drug may continue to engender significant levels of drug-appropriate responding following pretreatment with an antagonist because the discrimination is based on peripheral cues. Under this scenario, the conclusions regarding the neuropharmacological systems involved in mediating the behavioral effects of an abused stimulant may be erroneous even though the antagonist attenuated the central effects of the training drug.

Worth noting is that the discriminative-stimulus effects of commonly abused drugs overlap extensively when administered by various routes, including those used in the natural environment as well as ones designed to eliminate peripheral effects. Cocaine is typically inhaled (i.e., smoked), insufflated (i.e., snorted), or injected in the naturalistic environment. Cocaine is rarely ingested orally. In one study, five volunteers with histories of cocaine abuse learned to discriminate between 80 mg/70 kg oral cocaine and placebo [20]. A range of doses of oral and intranasal cocaine (20 to 120 mg/70 kg) as well as oral triazolam (0.25 and 0.50 mg/70 kg) was then tested. Oral and intranasal cocaine produced comparable dose-related increases in cocaine-appropriate responding. Triazolam produced low levels of cocaine-appropriate responding. The results of this study suggest that a route of administration not typically used in the natural environment may be employed as a model to assess the discriminative-stimulus effects of a commonly abused drug that produces robust peripheral effects.

4. Training Dose

The dose of the training drug is yet another important consideration. From a practical or ethical perspective, the minimum dose of the training drug should be used because participants will be exposed to it several times. Lower drug doses are, however, more

difficult to discriminate. In a series of studies conducted in the same laboratory, a total of 72 participants attempted to learn to discriminate between 10 mg oral d-amphetamine and placebo [10, 21–23]. Only 37 (51%) participants were able to acquire the discrimination. In a series of studies conducted in our laboratory, 39 participants attempted to learn to discriminate 15 mg oral d-amphetamine [24–28]. Thirty-three (85%) participants acquired the discrimination. Thus, a modest increase in the training dose resulted in a greater number of participants subsequently being able to acquire the d-amphetamine discrimination.

In addition to affecting the percentage of participants that are able to meet the discrimination criterion, training dose also influences subsequent discrimination performance. Preclinical laboratory experiments have shown that the dose-response curve for the training drug is shifted leftward in animals trained to discriminate lower versus higher drug doses [29].

We know of five published studies that explicitly examined the influence of training dose on discrimination performance in humans [30–34]. In one study, for example, participants learned to discriminate 10 mg d-amphetamine (i.e., low-dose group) or 20 mg d-amphetamine (i.e., high-dose group) [31]. After acquiring the d-amphetamine discrimination, a range of doses of d-amphetamine (1.25–20 mg) was tested to determine whether they shared discriminative-stimulus effects with the training dose. Participants in the low-dose group were more sensitive to the discriminative-stimulus effects of d-amphetamine as evidenced by a statistically significant leftward shift in the dose-response function (Figure 14-3).

Worth noting is that training dose did not affect responding on several of the subjective drug-effect questionnaires that were included as part of the human drug discrimination experiments described above [31, 32, 34]. For example, in the previous study conducted in our laboratory, d-amphetamine dose dependently increased scores on the A, BG, and MBG scales of the ARCI [31]. Neither the main effect of training dose nor the interaction of training dose and d-amphetamine dose attained statistical significance. Similarly, d-amphetamine dose dependently increased responses on eight items from an investigator-developed Drug-Effect Questionnaire (i.e., anxious/nervous; bad effects; feel the drug; good effects; improved performance; like the drug; stimulated; and feel like talking or socializing). The d-amphetamine dose-response function was shifted significantly leftward in the low-dose *versus* the high-dose group on only four of these items (i.e., improved performance; like the drug; stimulated; and feel like talking or socializing). These findings further support the notion that the discriminative-stimulus and subjective effects of drugs are not isomorphic. Future research is needed regarding the influence of training dose on the discriminative-stimulus effects of stimulants in humans. As discussed below, human drug discrimination procedures are sometimes used to elucidate the neuropharmacology of abused drugs. Future studies should determine whether training dose systematically influences the pharmacological selectivity of the discrimination. Preclinical studies have demonstrated that training dose alters the pharmacological selectivity of the cocaine discrimination [29]. In addition to dopamine, norepinephrine systems also appear to be involved in mediating the discriminative-stimulus effects of low cocaine doses (i.e., 3 mg/kg) [29], but not higher doses (i.e., 10 mg/kg) [35, 36].

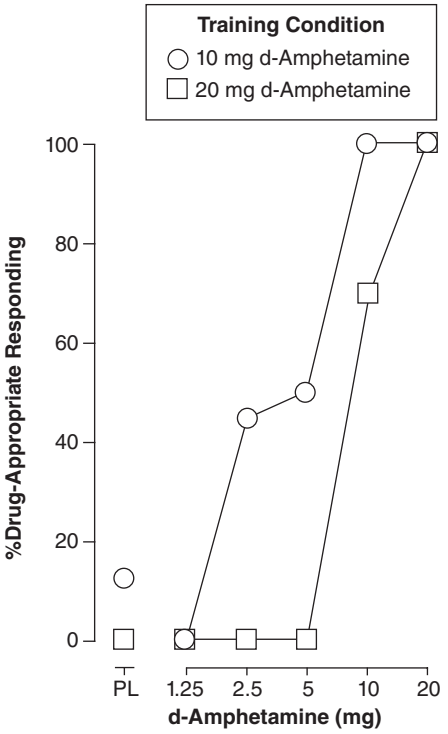

Figure 14-3. Dose effects for percent drug-appropriate responding on a point distribution procedure for participants that learned to discriminate a low (10 mg; circles) and high (20 mg; squares) dose of d-amphetamine. X-axes: dose in mg. Data points above "PL" designate placebo values. Y-axes: percent drug-appropriate responding. Data points show means of four participants for the low-dose group and five participants for the high-dose group. Error bars are omitted for clarity. Redrawn from Kollins and Rush (1999).

5. Instructions and Response Options

In a typical human drug discrimination experiment, participants are instructed verbally regarding their task. The use of verbal instructions is unique to the human drug discrimination paradigm and is advantageous for at least two reasons. First, the use of verbal instructions significantly reduces the number of experimental sessions that participants require to meet the discrimination criterion. Across a number of experiments that determined the discriminative-stimulus effects of stimulants (e.g., d-amphetamine, cocaine, methamphetamine, and methylphenidate), participants acquired the discrimination in 4–11 sessions [20, 27, 37, 38]. Of course, this number may be artificially low because some participants were excluded from further research participation if they were unable to acquire the discrimination in a predetermined number of sessions (i.e., 12 sessions). In preclinical drug discrimination experiments, by contrast, rodent and nonhuman primates often require 25 to 60 sessions to meet the discrimination criterion [39–42].

The second advantage is that human drug discrimination procedures allow investigators the unique opportunity to systematically assess the influence of different instructions on subsequent discrimination performance [43–45]. In many previous studies the investigators instructed participants that their task was to learn to discriminate between two drugs (e.g., Drug A versus Drug B). In other experiments, participants were instructed that their task was to decide whether they received Drug A or Not Drug A. An elegant pair of studies allowed the investigators to systematically compare the influence of these two different instruction sets on discrimination performance [44, 45]. In both studies, which were conducted in the same laboratory, volunteers with histories of opioid abuse learned to discriminate intramuscular hydromorphone (3 mg/70 kg). After acquiring the hydromorphone discrimination, dose-response functions were determined for hydromorphone, butorphanol, pentazocine, nalbuphine, and buprenorphine. These drugs have varying degrees of intrinsic efficacy at the mu and kappa opioid receptors, ranging from full agonists to antagonists [46]. In the experiment in which the Drug A versus Drug B instruction set was used, each drug substituted fully for hydromorphone [45]. In the study in which the Drug A versus Not Drug A instruction set was used, the results were more consistent with the intrinsic efficacy of these opiates for the mu and kappa opioid receptors [44]. Hydromorphone and buprenorphine dose-dependently increased drug-appropriate responding, and the highest dose of each drug substituted fully for the training dose (i.e., occasioned ≥80% drug-appropriate responding).

Butorphanol and nalbuphine did not completely substitute for hydromorphone at any of the doses tested. Pentazocine produced an inverted-U-shaped dose-response function. These findings illustrate that verbal instructions can influence subsequent discrimination performance and that the Drug A versus Not Drug A instruction set may be more sensitive to differences in the binding profile for a drug. In other words, the Drug A versus Not Drug A instruction set may increase the pharmacological selectivity of the discrimination. Future research should determine whether the Drug A versus Not Drug A instruction set increases the pharmacological selectivity of a stimulant discrimination.

6. Test Doses and Test Drugs

As mentioned above, in preclinical experiments, drug discrimination is pharmacologically sensitive in that larger drug doses generally engender increased drug-appropriate responding. Across a sufficient range of doses the dose-response curve for the training drug is typically quite steep, with at least one active dose producing minimal effect while at least one dose occasioning near maximal drug-appropriate responding. A steep, graded dose-response curve for the training drug can also be established in human participants using the drug discrimination paradigm assuming a sufficient range of doses is tested (Figures 14-2–14-4). Typically, a 6–16 fold range of doses of the training drug is required to accomplish this aim. In one previous experiment, participants learned to discriminate 30 mg oral methylphenidate [38]. After acquiring this discrimination, a range of doses of methylphenidate (0, 5, 10, 20, and 30 mg) was tested to establish a dose-response curve for the training drug. This range of doses engendered a steep, graded

dose-response curve. The lowest dose tested, 5 mg, engendered minimal drug-appropriate responding (i.e., ~21%), while the highest dose, 30 mg, engendered near maximal drug-appropriate responding (i.e., ~90%). A steep, graded dose-response for the training drug provides an ideal baseline to subsequently determine the influence of both behavioral (e.g., varying instructions) and pharmacological manipulations (e.g., pretreatment with an antagonist). Changes in the discriminative-stimulus effects of the training drug can then be quantified in terms of the magnitude of the shift of the dose response.

Occasionally a dose higher than the training dose is tested [8, 20, 47, 48]. As noted above, human drug discrimination procedures are sometimes used to elucidate the neuropharmacological mechanisms that mediate the discriminative-stimulus effects of the training drug based on the antagonists that shift the dose-response curve of the training drug rightward [7]. The inclusion of a dose higher than the training dose is important in these experiments to determine if the antagonism of the discriminative-stimulus effects of the training drug is surmountable.

Human drug discrimination procedures are also pharmacologically selective. As noted above, drugs from the same class as the training drug generally increase drug-appropriate responding as a function of dose, while drugs from different classes do not. When determining the pharmacological selectivity of the discriminative-stimulus effects of a drug at least two controls are necessary. The first control condition should be a compound that is pharmacodynamically and pharmacokinetically similar to the training drug (e.g., d-amphetamine in methamphetamine-trained participants). The *a priori* hypothesis would be that this compound would dose dependently increase drug-appropriate responding and at least one dose would substitute fully for the training dose (i.e., engender ≥80% drug-appropriate responding). The inclusion of a positive control is important in order to eliminate the possibility that participants will only emit drug-appropriate responding after receiving the training drug. Carefully considering the pharmacokinetics of the positive control compound is important so that participants do not base their discrimination on rate of onset. The second control condition should be a compound that is pharmacologically distinct from the training drug (e.g., triazolam in d-amphetamine-trained participants). The *a priori* hypothesis would be that this compound would not engender appreciable levels of drug-appropriate responding at any dose tested. Importantly, it must be possible to ascertain that the doses of this compound tested were behaviorally active. The inclusion of other behavioral indices (e.g., subjective drug-effect questionnaires or performance measures) is ideal for this reason. If, for example, triazolam did not increase subject ratings of sedation or impair performance in participants that had learned to discriminate d-amphetamine, the most parsimonious conclusion would be that insufficient doses were tested. The inclusion of a positive and negative pharmacological control is especially important if novel compounds are included and inferences are to be drawn concerning their behavioral pharmacological effect (i.e., abuse potential) relative to the training drug [11, 13].

7. Measurement Considerations

In general, there are two approaches used to express the primary outcome measure for a human discrimination experiment. Both approaches offer advantages, and neither is

"correct." The first approach is to determine the percentage of participants that identified a particular test condition as the training dose. This approach dichotomizes drug-appropriate responding (i.e., a participant identified a test dose as the training dose or he/she did not). The use of this approach results in a graded dose-response curve for the training drug [47, 49–51]. This approach also results in pharmacological selectivity in that compounds similar to the training drug generally increase the number of participants that identify the test dose as the training condition while compounds that are pharmacologically distinct from the training drug generally do not. Dichotomizing the outcome measure, however, requires the use of nonparametric inferential statistics, which are less powerful.

The second approach is to allow drug-appropriate responding to vary widely. In some human drug discrimination experiments, for example, participants distribute 100 points between two options (i.e., Drug A or Not Drug A, Drug A or Drug B, Red Drug or Blue Drug) depending on how certain he/she is of the identity of the administered drug [43, 45, 52]. Under this arrangement, the possible outcomes for drug-appropriate responding can vary from 0 to 100, inclusive. This approach is similar to using a 100-mm visual analog scale for measuring subjective drug effects and is amenable to the use of parametric inferential statistics (e.g., analysis of variance [ANOVA]).

8. Retrospective Analysis

Finally, human drug discrimination experiments typically involve a relatively small number of participants. Delineating the relative contribution of individual differences to the discriminative-stimulus effects of drugs is difficult with small sample sizes. The contribution of individual differences to the discriminative-stimulus effects of drugs has been examined using retrospective analyses [53–55]. Data are combined from multiple experiments in these analyses. In order to conduct such analyses, the experimental conditions must remain relatively constant. For example, the training dose, as well as the range of doses of the training drug, should be held constant across each of the experiments if the conduct of retrospective analyses is planned. As another example, the acquisition criterion should be held constant in order to ensure that all participants have similar recent pharmacological histories before entering a test phase.

In a recently published report, the effects of gender on the discriminative-stimulus effects of d-amphetamine were assessed retrospectively [55]. Gender was selected as the variable of interest because women may be more vulnerable to stimulant-use disorders than men [56, 57]. Data were combined from six experiments conducted in same laboratory that used identical procedures and measures to examine the discriminative-stimulus effects of d-amphetamine following pretreatment with potential pharmacotherapies for stimulant-use disorders. Across these six separate experiments, 13 women and 14 men learned to discriminate 15 mg oral d-amphetamine. After acquiring the discrimination, the effects of a range of doses of d-amphetamine (0, 2.5, 5, 10, and 15 mg), alone and in combination with other drugs, were assessed. Only data from sessions in which d-amphetamine was administered alone were included in this analysis. d-Amphetamine functioned as a discriminative-stimulus and dose-dependently

increased drug-appropriate responding. Women and men did not differ in their ability to discriminate d-amphetamine.

The results of these retrospective analyses suggest that women and men are not differentially sensitive to the discriminative-stimulus effects of d-amphetamine. These findings along with those from other reports suggest that individual differences may not contribute significantly to the discriminative-stimulus effects of drugs [58]. Alternatively, drug discrimination procedures may minimize individual differences in terms of responses to stimulants. As described above, human drug discrimination procedures provide participants with similar recent behavioral and pharmacological histories. As a result, the contribution of individual differences might be reduced or eliminated in this paradigm. Human drug discrimination procedures may be especially useful when studying a heterogeneous sample.

9. Summary

This section discussed some methodological issues that must be considered when designing and conducting a human drug discrimination experiment. This review was not intended to be exhaustive nor was its purpose to provide a recipe for the conduct of a human drug discrimination experiment. The considerations discussed included: 1) whether the use of human drug discrimination procedures is appropriate for addressing the experimental question; 2) route of administration; 3) training dose; 4) instructions and response options; 5) test doses and drugs; 6) measurement issues; and 7) standardizing procedures across experiments so that data can be combined for retrospective analyses. These methodological considerations were generally discussed in the context of human drug discrimination experiments involving amphetamines. However, the information gleaned from this review is probably germane to all human drug discrimination studies regardless of the pharmacological class of the medications being studied.

C. USING HUMAN DRUG DISCRIMINATION TO ELUCIDATE THE NEUROPHARMACOLOGY OF AMPHETAMINES

Abused stimulants produce their behavioral and physiological effects *via* interaction with monoamine transporters (dopamine [DA], serotonin [5-HT], and norepinephrine [NE]) [59–62]. Based on in vitro studies, stimulants can be broadly categorized into two groups by their mechanism of action at these transporters. Amphetamines act as substrates for monoamine transporters and are transported into the nerve terminal [62]. Amphetamines promote the release of these monoamines into the synapse by preventing the accumulation of neurotransmitters in storage vesicles, and also by carrier-mediated exchange [62]. Amphetamines also usually function as transporter blockers, although they are less potent at inhibiting reuptake compared to their ability to act as transporter substrates [63]. Cocaine, by contrast, binds to monoamine transporters and prevents the reuptake of these monoamines back into the presynaptic terminal, but is not transported.

In a typical preclinical behavioral neuropharmacology experiment, animals are initially trained to discriminate an abused stimulant like methamphetamine. After acquiring the discrimination, a dose-response curve is established for the training drug. This dose-response curve is then re-determined following pretreatment with pharmacologically specific antagonists. Inferences are then made regarding the neuropharmacological mechanisms that mediate the discriminative-stimulus effects of an amphetamine based on the antagonists that shift the dose-response curve rightward.

Preclinical behavioral pharmacology studies have implicated a prominent role of central dopamine systems in mediating the internal stimulus effects of amphetamines. In one study, squirrel monkeys were trained to discriminate intramuscular methamphetamine (0.3 mg/kg) [64]. Methamphetamine (0.03–0.3 mg/kg) dose-dependently increased drug-appropriate responding. Pretreatment with dopamine receptor blockers (i.e., SCH39166 [(-)-trans-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo[d]naphtho-[2,1-b]-azepine)], remoxipride and nemonapride) antagonized the discriminative effects of methamphetamine.

The role of central dopamine systems in mediating the behavioral effects of amphetamines in humans has been explored using subjective-effect questionnaires [65]. In these studies participants were administered a range of doses of a stimulant (e.g., (+)amphetamine or methamphetamine) alone and following pretreatment with a dopamine antagonist. Inferences regarding the neuropharmacological mechanism that mediates the effects of amphetamine were made depending on the pretreatment drugs that alter the subjective drug effects. Driven by the tenet that the discriminative-stimulus effect of drugs in nonhuman laboratory animals is a model of subjective effects in humans, the central hypothesis of these human laboratory studies is that dopamine antagonists should attenuate or block the subjective effects of amphetamines.

The results of human laboratory studies have not convincingly demonstrated the involvement of central dopamine systems in mediating the subjective effects of amphetamines in humans [65]. In a series of studies from the same laboratory, the subjective effects of (+)amphetamine (10–20 mg) were assessed following pretreatment with the dopamine antagonists pimozide (1–8 mg) and fluphenazine (3–6 mg) [66–68]. (+)-Amphetamine produced prototypical positive subjective effects (e.g., increased ratings of drug liking, good effects and stimulated). Neither pimozide nor fluphenazine altered the subjective effects of (+)amphetamine. In yet another study conducted by these investigators, the subjective effects of methamphetamine (0 or 20 mg) were assessed following pretreatment with haloperidol (0 or 3 mg), a D_2 antagonist, or risperidone (0 or 0.75 mg), an atypical antipsychotic that is a mixed DA/5-HT antagonist [69]. Methamphetamine produced prototypical stimulant-like subject-rated effects. Neither haloperidol nor risperidone significantly altered these effects.

Drug discrimination procedures have been used rather infrequently in human laboratory studies designed to elucidate the neuropharmacological mechanisms in mediating the behavioral effects of amphetamines. However, the extant literature suggests that the concomitant use of a drug discrimination procedure and subjective-effect questionnaires produce results that are consistent with the notion that central monoamine systems mediate the behavioral effects of amphetamines in humans, namely dopamine and serotonin. In a series of unpublished studies, participants learned to discriminate

15 mg oral (+)amphetamine [70]. After acquiring the discrimination, the effects of a range of doses of d-amphetamine (0, 2.5, 5, 10, and 15 mg), alone and following pretreatment with fluphenazine (0, 3 and 6 mg), were assessed. D-Amphetamine functioned as a discriminative stimulus and produced prototypical stimulant-like subjective effects. The low dose of fluphenazine, 3 mg, did not alter the behavioral effects of d-amphetamine in the four participants that completed this study. The high dose of fluphenazine, 6 mg, produced a robust rightward shift in the d-amphetamine dose-response curve in the one participant that completed the study (Figure 14-4; top panel). While these data should be viewed cautiously because only a single participant was involved, they are consistent with the notion that central dopamine systems mediate the behavioral effects of amphetamines in humans. In another experiment, eight volunteers learned to discriminate 15 mg oral d-amphetamine [26]. D-Amphetamine alone functioned as a discriminative stimulus and produced prototypical stimulant-like subjective effects. Risperidone pretreatment significantly attenuated the discriminative-stimulus and some of the subjective effects (Figure 14-4; middle panel). The results of these experiments are consistent with a role of dopamine and serotonin in the behavioral effects of amphetamines. Collectively, the results of these studies suggest that the concomitant use of drug discrimination procedures and subjective-effect questionnaires may yield results that are more consistent with the pharmacology of amphetamines.

The results of several recent publications that assessed the behavioral effects of amphetamines following pretreatment with a partial dopamine agonist also suggest that the combined use of drug discrimination procedures and subjective-effect questionnaires yields results that are consistent with the notion that central monoamine systems mediate the behavioral effects of stimulants in humans [24, 28, 71, 72]. Partial agonists are receptor ligands with significant receptor affinity, but low intrinsic activity. Theoretically, these drugs may be expected to have both agonist and antagonist effects. Under conditions of low neurotransmitter tone, as is observed for dopamine (DA) during initial abstinence from chronic stimulant administration [73], a partial agonist should produce some receptor stimulation, thereby functioning as an agonist. In contrast, a partial agonist may act as an antagonist when there are higher levels of neurotransmitter present in the synapse, as would occur following stimulant administration.

In a recent study, the effects of intravenous methamphetamine (0, 15, and 30 mg) were assessed in separate groups of participants maintained on placebo (N = 8) or aripiprazole (15 mg/day for two weeks; N = 8) [71]. Aripiprazole is an atypical antipsychotic that is a partial agonist at D_2 receptors [74]. As expected, methamphetamine produced prototypical subjective effects (e.g., ratings of high and stimulated) that were an orderly function dose in both groups. The subjective effects of methamphetamine were, however, significantly greater in the aripiprazole-maintained participants. In another experiment, six volunteers learned to discriminate 10 mg oral methamphetamine [72]. After acquiring the discrimination, the effects of a range of doses of methamphetamine (0, 2.5, 5, 10, and 15 mg), alone and following pretreatment with aripiprazole (0 and 20 mg), were assessed. Methamphetamine functioned as a discriminative stimulus and produced prototypical stimulant-like subjective effects. The three highest doses of methamphetamine (i.e., 5, 10, and 15 mg) increased drug-appropriate

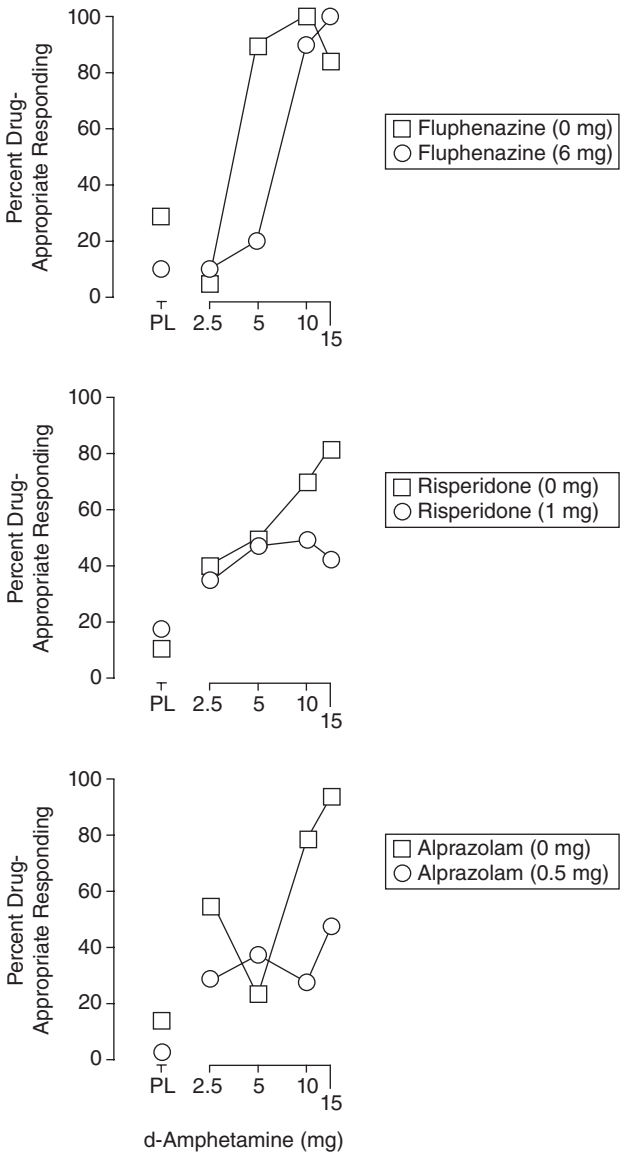

Figure 14-4. Percent drug-appropriate appropriate responding for placebo, d-amphetamine alone, fluphenazine, risperidone or alprazolam alone, d-amphetamine-fluphenazine, d-amphetamine-risperidone or d-amphetamine-alprazolam combinations. X-axes: d-Amphetamine Dose. Data points above PL represent values when the doses of fluphenazine, risperidone or alprazolam were administered in combination with 0 mg d-amphetamine. Connected data points above 2.5, 5, 10, and 15 represent the effects of the d-amphetamine dose administered in combination fluphenazine, risperidone or alprazolam (0 mg [squares] or fluphenazine, risperidone or alprazolam [circles]). Data points show means of one to eight participants. The middle and bottom panels were redrawn from Rush et al. (2003 and 2004, respectively).

responding significantly above placebo levels following pretreatment with placebo or aripiprazole. However, relative to placebo pretreatment, drug-appropriate responding was significantly lower after aripiprazole pretreatment. Similar effects were observed on several subjective effects items (e.g., ratings of Talkative-Friendly and Active-Alert-Energetic). Of course, these discrepant findings could be attributable to the use of different methods (i.e., acute versus chronic aripiprazole dosing; different aripiprazole doses; administrating methamphetamine orally versus intravenously; or recreational users versus methamphetamine-dependent patients).

Finally, studies that pretreated participants with an indirect dopamine antagonist further suggest that the combined use of human drug discrimination procedures and subjective-effect questionnaires may be an effective methodological strategy for elucidating the neuropharmacology of amphetamines. Neuroanatomical and neurochemical data suggest that central dopamine systems are under the inhibitory control of γ-aminobutyric-acid (GABA) systems. First, approximately 80% of neurons in the nucleus accumbens are GABAergic [75]. Second, $GABA_A$ receptors are located on the dopamine cell bodies in the ventral tegmental area, while $GABA_B$ receptors are located on the interneurons [76, 77]. Third, systemic injections of benzodiazepines (i.e., diazepam and midazolam) reduce the release of dopamine in the nucleus accumbens [78, 79]. Fourth, injections of flurazepam, a benzodiazepine, into the nucleus accumbens attenuate dopamine transmission [80]. Fifth, lorazepam, another benzodiazepine, and γ-vinyl GABA, an irreversible GABA-transaminase inhibitor, attenuate cocaine-induced increases in dopamine levels in the striatum and nucleus accumbens [81, 82].

Consistent with the findings of the neuroanatomical and neurochemical studies described above, the results of preclinical behavioral pharmacology studies suggest that $GABA_A$ receptor modulators attenuate the discriminative-stimulus effects of amphetamines [83]. In this study, 48 rats were trained to discriminate 1.0-mg/kg d-amphetamine. After acquiring the discrimination, a range of doses of d-amphetamine (0–1.0 mg/kg) was tested alone and following pretreatment with midazolam (0–0.2 mg/kg), a high-affinity $GABA_A$ receptor modulator. D-Amphetamine alone dose dependently increased percent drug-appropriate responding. Midazolam alone did not occasion significant percent drug-appropriate responding, but dose dependently attenuated the discriminative-stimulus effects of d-amphetamine.

We know of two human laboratory studies in which the behavioral effects of d-amphetamine were assessed alone and following pretreatment with a high-efficacy $GABA_A$ receptor modulator [27, 84]. In the first experiment, the combined effects of oral d-amphetamine (0 and 20 mg/70 kg) and triazolam (0 and 0.25 mg/70 kg) were assessed in 20 healthy, non-drug-abusing individuals [84]. D-Amphetamine alone produced a constellation of stimulant-like subjective effects (e.g., increased ratings of Good Effects), while triazolam alone produced sedative-like self-reported drug effects (e.g., increased ratings of sleepiness). Triazolam generally did not attenuate the subjective effects of d-amphetamine and in several instances augmented them. In the other experiment, six healthy humans learned to discriminate 15-mg oral d-amphetamine [27]. After acquiring the discrimination, the effects of d-amphetamine (0, 2.5, 5, 10, and 15 mg), alone and following pretreatment with alprazolam (0 and 0.5 mg), a high-efficacy $GABA_A$ receptor modulator, were assessed. d-Amphetamine alone functioned

as a discriminative stimulus and produced stimulant-like subjective effects (e.g., increased ratings of Good Effects). These effects were generally a function of dose. Alprazolam alone did not occasion d-amphetamine-appropriate responding, nor did it increase ratings of sedation or impair performance. Alprazolam significantly attenuated the discriminative effects of d-amphetamine (Figure 14-4, bottom panel), as well as some of the subjective effects. The results of this study further suggest that the use of both drug discrimination and subjective-effect questionnaires may yield results that are consistent with the pharmacology of amphetamines.

In summary, drug discrimination procedures have been used rather infrequently in human laboratory studies designed to elucidate the neuropharmacological mechanisms in mediating the behavioral effects of amphetamines. The extant literature suggests that the concomitant use of drug discrimination procedures and subjective-effect questionnaires may yield results that are more consistent with the pharmacology of amphetamines. The results of human laboratory studies that used both a drug discrimination procedure and subjective-effect questionnaires suggest that central monoamine systems, namely dopamine and serotonin, mediate the behavioral effects of amphetamines. The results of these human behavioral pharmacology studies are, of course, concordant with those from *in vivo* and preclinical experiments. Future human drug discrimination studies are needed to more fully characterize the neuropharmacological mechanisms that mediate the internal stimulus effects of amphetamines in humans. For example, these studies might test norepinephrine antagonists. As discussed below, elucidating the neuropharmacological mechanisms that mediate the internal stimulus effects of amphetamines in humans could have implications for identifying putative pharmacotherapies to manage amphetamine-use disorders.

D. THE FUTURE OF HUMAN DRUG DISCRIMINATION

The extant literature reviewed above suggests that the concomitant use of a human drug discrimination procedure and subjective-effect questionnaires produces results that align more consistently with the known neuropharmacology of amphetamines. The studies that used both a drug discrimination procedure and subjective-effect questionnaires suggest central monoamine systems, namely dopamine and serotonin, mediate the behavioral effects of amphetamines, which, of course, is concordant with *in vivo* and preclinical experiments. One extension of this work would be to determine if the combined use of a drug discrimination procedure and subjective-effect questionnaires produces results that are concordant with the known pharmacological mechanisms of other drugs of abuse.

A series of studies conducted in the same laboratory suggest that the combined use of a human drug discrimination procedure and subjective-effect questionnaires produced results that are concordant with the known neuropharmacological mechanisms of opioids. As reviewed above, in one study volunteers with histories of opioid abuse learned to discriminate hydromorphone (3 mg/70 kg) [44]. After acquiring the hydromorphone discrimination, dose-response functions were determined for hydromorphone (0.125–4 mg/70 kg), butorphanol (0.375–6 mg/70 kg), pentazocine

(4–64 mg/70 kg), nalbuphine (1.5–24 mg/70 kg), and buprenorphine (0.055–0.9 mg/70 kg). These drugs have varying degrees of intrinsic efficacy at the mu and kappa opioid receptors, ranging from full agonists to antagonists [46]. Hydromorphone and buprenorphine dose-dependently increased drug-appropriate responding, and the highest dose of each drug substituted fully for the training dose (i.e., occasioned ≥80% drug-appropriate responding). Butorphanol and nalbuphine did not completely substitute for hydromorphone at any of the doses tested. Pentazocine produced an inverted-U-shaped dose-response function. Hydromorphone and buprenorphine increased subject ratings of Drug Liking and Good Effects as an orderly function of dose. Pentazocine produced an inverted U-shaped dose-response function on these ratings. Butorphanol and nalbuphine did not significantly increase ratings of Drug Liking or Good Effects. In a previous study conducted in this laboratory that employed only subjective-effect questionnaires, intramuscular hydromorphone (5.25 mg total cumulative dose) and butrophanol (105 mg total cumulative dose) produced comparable increases in ratings of Drug Liking and Good Effects [85]. In yet another study conducted in this laboratory that employed only subjective-effect questionnaires, intramuscular hydromorphone (1.5–6 mg/70 kg) and butorphanol (1.5–12 mg/70 kg) produced comparable dose-related increases in ratings of Drug Liking and Good Effects [86]. Thus, as was the case with central nervous system stimulants, these results collectively suggest that the combined use of a human drug discrimination procedure and subjective-effects questionnaires yield results that are consistent with the neuropharmacology of opioids. Future research should determine if the combined use of a human drug discrimination procedure and subjective-effect questionnaires yield results that are consistent with the neuropharmacology of other drugs of abuse such as sedatives.

Because of their neuropharmacological sensitivity, human drug discrimination procedures might be used to determine the initial efficacy of putative pharmacotherapies for amphetamine dependence. Methamphetamine abuse and dependence is a significant public-health concern. The number of individuals reporting recent use of methamphetamine has remained relatively stable over the past four years with approximately 731,000 Americans reporting past-month use in 2006 [87]. Rates of primary treatment admissions for methamphetamine nearly doubled between 2000 and 2004 [88]. Because of these epidemiological data, identifying an effective pharmacotherapy for amphetamine dependence is a public health priority [89]. An effective pharmacotherapy for methamphetamine dependence has not yet been identified despite 15 years of research. Human laboratory experiments designed to determine the efficacy of a putative pharmacotherapy typically administer a range of doses of methamphetamine following pretreatment or maintenance on varying doses, including placebo, of the candidate medication [90]. A putative pharmacotherapy that alters the discriminative and/or subjective effects of methamphetamine should then be tested in a clinical trial. Double blind, placebo-controlled, randomized trials are, of course, the gold standard of clinical research. Clinical trials, however, are costly, time consuming, and labor intensive, and should be reserved for only the most promising medications (i.e., compounds that alter at least some of the behavioral effects under controlled laboratory conditions) [90].

The medications studied are often chosen based on the neuropharmacological mechanisms that mediate the behavioral effects of amphetamines. As reviewed above,

amphetamines act as substrates for monoamine transporters and are transported into the nerve terminal where they promote the release of these monoamines into the synapse by preventing the accumulation of neurotransmitter in storage vesicles, and also by carrier-mediated exchange [62]. In vivo, preclinical, and, as reviewed above, human drug discrimination studies suggest that central monoamine systems, most notably dopamine and serotonin, mediate the internal stimulus effects of amphetamines.

Because of the involvement of monoamine systems in mediating the internal stimulus effects of amphetamines, there has been considerable interest in testing medications that function as anatagonists in these systems. The premise of this approach is that treating patients with an antagonist will block the behavioral effects of amphetamine (e.g., discriminative or subject-rated effects), thereby leading to a reduction in drug taking. Antagonist therapies like mecamylamine and naltrexone are somewhat effective for nicotine and opioid dependence, respectively [91–93]. We know of only a single antagonist, risperidone, tested as putative pharmacotherapy for amphetamine dependence in both a human drug discrimination study and a clinical trial [26, 94]. Risperidone is an antipsychotic that is a mixed D_2 and 5-HT_2 antagonist [95]. As described above, eight volunteers learned to discriminate 15 mg oral d-amphetamine [26]. D-Amphetamine alone functioned as a discriminative stimulus and dose-dependently increased drug-appropriate responding. Risperidone pretreatment significantly attenuated the discriminative-stimulus effects of d-amphetamine (Figure 14-4; middle panel). In an open-label pilot study, eight methamphetamine-dependent patients were maintained on risperidone (3.6 mg average daily dose) for four weeks [94]. These patients reduced their methamphetamine use from 13 out of 30 days prior to starting the trial to less than one day during the trial. These results suggest that compounds that attenuate the discriminative-stimulus effects of amphetamine may reduce drug use in the naturalistic environment.

We know of another antipsychotic, aripiprazole, tested as a putative pharmacotherapy for amphetamine dependence using a human drug discrimination procedure and in a clinical trial [24, 28, 72, 96]. Aripiprazole is an atypical antipsychotic that is a partial agonist at D_2 receptors [74]. Aripiprazole also has significant affinity for, and varying degrees of intrinsic efficacy at, the 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 receptor subtypes [97]. A series of human drug discrimination experiments and clinical trials have examined the efficacy of aripiprazole and as putative pharmacotherapy for amphetamine-use disorders [24, 28, 72, 96]. In the drug discrimination experiments, participants with a history of non-therapeutic stimulant use learned to discriminate oral d-amphetamine (15 mg) [24, 28] or methamphetamine (10 mg) [72]. After acquiring the discrimination as described above, the effects of a range of doses of d-amphetamine and methamphetamine (0, 2.5, 5, 10, and 15 mg), alone and in combination with aripiprazole (0, 10, or 20 mg), were assessed. D-Amphetamine and methamphetamine alone functioned as a discriminative-stimulus and dose dependently increased drug appropriate responding in each of these experiments. The high dose of aripiprazole, 20 mg, attenuated the discriminative-stimulus effects of d-amphetamine and methamphetamine while the low dose failed to attenuate the discriminative-stimulus effects of d-amphetamine. In the clinical trial, amphetamine-dependent drug-injecting patients were

randomly assigned to receive 15-mg/day aripiprazole (N = 19) or placebo (N = 17) for 20 weeks [96]. Aripiprazole failed to reduce the percentage of amphetamine-positive urine specimens. Definitive conclusions regarding the ability of aripiprazole to attenuate the discriminative-stimulus effects of amphetamine and its efficacy to reduce drug use in the naturalistic environment are difficult due to the use of different doses across the laboratory studies and the clinical trial.

In a recent drug discrimination experiment, cocaine-dependent participants learned to discriminate oral 150 mg oral cocaine [98]. After acquiring the discrimination, the effects of a range of doses of oral cocaine (0, 25, 50, 100, and 200 mg), alone and in combination with aripiprazole (0 or 15 mg), were assessed. Oral cocaine alone functioned as a discriminative-stimulus and dose dependently increased drug appropriate responding. This dose of aripipriazole failed to attenuate the discriminative-stimulus effects of oral cocaine. These results suggest that a dose of aripiprazole that fails to attenuate the discriminative-stimulus effects of stimulants will be ineffective in decreasing drug use in the naturalistic environment [96, 98]. A human laboratory study is needed that tests 15 mg aripiprazole in amphetamine-trained humans before definitive conclusions regarding its ability to attenuate the discriminative-stimulus effects of amphetamine and reduce drug use in the naturalistic environment can be ascertained.

Putative agonist replacement therapies have also been tested using a human drug discrimination procedure and in a clinical trial. The premise of replacement therapy is that treating patients with an agonist presumably suppresses withdrawal and produces tolerance to the behavioral effects (i.e., discriminative) of methamphetamine, thereby leading to reduced drug taking. Agonist replacement therapies like nicotine replacement products and methadone are amongst the most effective medications for managing nicotine and opioid dependence, respectively [99, 100].

Few preclinical or human laboratory studies have examined the effects of potential agonist-replacement therapies on the discriminative effects of amphetamine. In one preclinical study, squirrel monkeys were trained to discriminate intramuscular methamphetamine (0.3 mg/kg) [39]. Methamphetamine (0.01–0.3 mg/kg) dose-dependently increased drug-appropriate responding. GBR12909 (1-[2-[bis(4-fluorophenyl)methoxy] ethyl]-4-3(phenylpropyl)-piperazine) (1.0–17.8 mg/kg), a potent dopamine uptake blocker, also dose-dependently increased drug-appropriate responding. Pretreatment with GBR12909 (1.0–3.0 mg/kg) shifted the methamphetamine dose-response curve leftward. In an elegant experiment various dopamine uptake inhibitors and dopamine releasers were tested to determine whether they altered the discriminative-stimulus effects of cocaine in rats [101]. Following acquisition of the cocaine discrimination, the dopamine releasers methamphetamine, d-amphetamine, methcathinone, cathinone, fencamfamine, and phentermine as well as the dopamine uptake inhibitors GBR12909, WIN 35,428, methylphenidate, indatraline, nomifensine, and mazindol were tested in combination with cocaine. Pretreatment with either dopamine uptake inhibitors or dopamine releasers shifted the cocaine dose-response curve leftward. The dopamine releasers, specifically d-amphetamine and methamphetamine, were more potent in shifting the curve leftward than the dopamine uptake inhibitors. The authors concluded that perhaps, while counterintuitive, the most promising agonist replacement therapies

would be those that shift the cocaine dose-response curve furthest to the left when administered acutely.

We know of two compounds, d-amphetamine and bupropion, tested as potential agonist replacement therapies for amphetamine dependence in both a human drug discrimination study and a clinical trial. D-Amphetamine is a potent dopamine releaser [62]. In a human laboratory experiment, five volunteers learned to discriminate 10 mg oral methamphetamine [102]. After acquiring the discrimination, the effects of a range of doses of methamphetamine (0, 1.25, 2.5, 5, and 10 mg), alone and following pretreatment with (+)amphetamine (0 and 15 mg), were assessed. Methamphetamine functioned as a discriminative stimulus and dose-dependently increased drug-appropriate responding. D-Amphetamine pretreatment shifted the methamphetamine dose-response curve upward and to the left. In the seminal clinical trial, 63 amphetamine-dependent patients prescribed d-amphetamine (i.e., maximum 40 mg/day) were compared to 25 matched controls [103]. There were statistically significant differences between the d-amphetamine-treated patients and controls in terms of illicit drug use and clinic attendance. Similar results have been observed with (+)amphetamine and structurally related compounds (e.g., methylphenidate) [96, 104–106]. These results suggest that potential agonist therapies that accentuate the discriminative-stimulus effects of amphetamine may reduce drug use in the naturalistic environment. These results also further support the notion that human drug discrimination procedures may be well suited for determining the initial efficacy of potential agonist replacement therapies for methamphetamine dependence.

Bupropion is a weak dopamine reuptake inhibitor that has some stimulant-like effects [13, 107]. In a human laboratory experiment, five volunteers learned to discriminate 10 mg oral methamphetamine [108]. After acquiring the discrimination, the effects of a range of doses of methamphetamine (0, 1.25, 2.5, 5, and 10 mg), alone and following pretreatment with bupropion (0 and 150 mg), were assessed. Methamphetamine functioned as a discriminative stimulus and dose-dependently increased drug-appropriate responding. Bupropion pretreatment did not alter the discriminative-stimulus effects of methamphetamine to a significant degree. Bupropion has also been tested in double blind, placebo-controlled clinical trials [109, 110]. In the first trial, methamphetamine-dependent patients were randomly assigned to receive placebo (N = 72) or sustained-release bupropion (150 mg BID) (N = 79) [109]. In the second trial, methamphetamine-dependent patients were randomly assigned to receive placebo (N = 37) or sustained-release bupropion (150 mg BID) (N = 36) [110]. Drug urine tests were conducted 2–3 times per week and were the primary outcome measure in both of these trials. The bupropion- and placebo-treated patients did not differ significantly in terms of amphetamine-negative urine samples. These results suggest that potential agonist therapies that do not accentuate the discriminative-stimulus effects of amphetamine may be ineffective clinically and further support the notion that human drug discrimination procedures may be well suited for determining the initial efficacy of potential agonist replacement therapies for methamphetamine dependence.

In summary, the available human laboratory and clinical literature suggest that human drug discrimination procedures may be well suited for determining the initial efficacy of putative pharmacotherapies for methamphetamine dependence. The antipsychotic risperidone attenuated the discriminative-stimulus effects of d-amphetamine in a

human laboratory experiment and reduced drug use in the naturalistic environment [26, 94]. Another antipsychotic, aripipiprazole (15 mg), failed to attenuate the discriminative-stimulus effects of cocaine and did not reduce stimulant use in a clinical trial [96, 98]. While perhaps counterintuitive, an effective agonist replacement therapy may enhance the discriminative-stimulus effects of methamphetamine. D-Amphetamine, a potent dopamine releaser, augmented the discriminative-stimulus effects of methamphetamine and reduced drug use in a clinical trial [102, 103]. Bupropion, a weak dopamine uptake blocker, by contrast, did not augment the discriminative-stimulus effects of methamphetamine nor did it reduce drug use in clinical trials [89, 108, 110]. While the existing literature suggests that human drug discrimination procedures may be a valid assay for determining the initial efficacy of putative pharmacotherapies for amphetamine dependence, there are at least two limitations with some of the clinical trials described above. First, some of the clinical trials described above were rather small. In the clinical trials that assessed the efficacy of risperidone and aripiprazole for reducing drug use, for example, only 8–19 amphetamine-dependent patients were treated with the medication of interest [94, 96]. Second, some of the trials did not utilize a double-blind, placebo-controlled design, which, of course, is the gold standard of clinical research. In the seminal clinical trial that assessed the efficacy of d-amphetamine for reducing methamphetamine use, the experimental group (i.e., amphetamine-dependent patients treated with a maximum 40 mg/day d-amphetamine) was compared to untreated matched controls rather than placebo-treated patients [103]. As another example, the clinical trial that demonstrated that risperidone reduces methamphetamine use employed an open-label design [94]. Open-label designs may be prone to false-positive results. While limited, these are the only clinical data available to our knowledge that tested the same compounds that were used in human drug discrimination experiments. The conduct of larger, double blind placebo-controlled clinical trials with these compounds in the future will allow the predictive validity of the human drug discrimination procedure to be established more definitively. Determining the predictive validity of human laboratory procedures in general, and human drug discrimination procedures in particular, is important because laboratory studies can be conducted more rapidly and efficiently than clinical trials. Definitively establishing the predictive validity of human drug discrimination will ultimately determine the public health relevance of this rigorous behavioral assay.

Acknowledgments Preparation of this manuscript was supported, in part, by grants from the National Institute on Drug Abuse (DA010325, DA017711, DA020429, DA021155, DA025591, and DA025032) awarded to Craig R. Rush.

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15

NICOTINE DISCRIMINATION IN HUMANS

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

A. INTRODUCTION

Nicotine produces interoceptive stimulus effects that may help explain its reinforcing efficacy [1, 2]. The most common approach to assessing these effects in humans is via self-report questionnaires of subjective effects, including mood and more specific drug effects (e.g., Addiction Research Center Inventory, or ARCI), as discussed in detail elsewhere [3]. Typical subjective effects of nicotine are increases in arousal, "head rush," and alertness, although the magnitude, and even direction, of such effects depend on many conditions [4].

However, there are several reasons interoceptive stimulus effects of nicotine are not completely captured by self-report measures. First, subjective reports only overlap, and are not synonymous, with interoceptive stimulus effects [5], and such effects may not be adequately characterized with self-report measures alone. Second, many self-report measures require good language comprehension and often use terms unfamiliar to some drug users. Third, and perhaps most critically, the subjective effects being reported by subjects cannot be independently verified, by their very nature (see also Chapter 1). This problem raises the possibility that self-report responses are not reliable indices of actual drug stimulus effects but reflect the subject's expectations of the effects of the substance [6] or are otherwise biased.

By contrast, the behavioral drug discrimination procedure does not suffer from these particular problems and so provides a valuable approach to reliably assess interoceptive stimulus effects of nicotine in humans. Behavioral drug discrimination is essentially the only method for the study of interoceptive drug effects in nonhuman animals, which lack verbal ability. Although other methods are available and commonly used in human studies, discrimination testing in humans is nevertheless an important addition to understanding a drug's effects. Nicotine discrimination studies in humans provide a means to determine the extent to which discrimination behavior findings in animals generalize to humans [7]. In addition, since drug discrimination is often viewed as an animal model of subjective effects in humans, directly comparing discriminative stimulus and subjective effects is necessary and can only be done with humans [8]. Finally, drug discrimination results can indicate the neural sites of action of drugs [9], which may aid in medication development. At the same time, compared to self-report measures, the drug discrimination procedure has its own shortcomings when used in humans, such as requiring extensive time for training and testing and being less sensitive to qualitative differences in a drug's stimulus effects [10]. Thus, use of drug discrimination procedures may not be suitable for all studies of nicotine's interoceptive stimulus effects in humans, but the procedure merits greater attention given its utility [e.g., 11].

This chapter first outlines the general methods of nicotine discrimination in humans, identifying key obstacles to overcome in controlling nicotine administration. Next is a section discussing some of the parameters of nicotine discrimination, including tolerance to discrimination, the threshold (or minimum dose) necessary for discrimination, and central mediation of discrimination behavior. The final section describes individual differences and environmental factors that may moderate nicotine discrimination

behavior. These factors are of interest in their own right but also should be controlled in research examining other influences on nicotine discrimination. Studies from the author's program of research using nicotine nasal spray will be emphasized, although research by other investigators will be noted where relevant.

B. BASIC METHODS OF NICOTINE DISCRIMINATION RESEARCH IN HUMANS

1. Dosing Issues

Although animal studies of nicotine discrimination date from the 1960s [12], research on nicotine discrimination in humans did not occur until the 1980s, and programmatic study did not begin until the 1990s. One reason for this delay in research interest was the uncertain importance of nicotine in reinforcing cigarette smoking until the 1980s [13]. However, the most significant obstacle to studying nicotine discrimination in humans was the lack of methods of administering nicotine doses in a controlled manner, a requirement of any study on drug discrimination. The main method of consuming nicotine is cigarette smoking, which allows wide variability in puff topography, or the intensity and pattern of smoke inhalation, and therefore variability in nicotine dose [14]. Even when cigarettes differing sharply in stated nicotine yield are used to manipulate nicotine dose, the actual delivery of nicotine to smokers may not vary because of the ease with which smokers can alter smoking intensity per puff [15]. Moreover, because cigarette smoke contains thousands of chemical constituents other than nicotine [16], some of which may be psychoactive, the discriminative stimulus effects of nicotine may be confounded with those of these other constituents. Use of tobacco smoking also confounds discrimination of cigarettes based on differences in the interoceptive effects of nicotine versus differences in peripheral or sensory effects of smoking those cigarettes, such as harshness or taste [17]. As a consequence of these problems, the earliest studies of nicotine discrimination in humans using cigarettes varying in nicotine yield could not verify that the discrimination was based on nicotine effects versus other cigarette characteristics [17, 18].

Nicotine replacement therapy (NRT) via gum was developed in the 1980s, providing the first practical method to administer nicotine isolated from other tobacco constituents to humans. NRT patch and other formulations followed in the 1990s. However, these alternatives introduced other problems in their use in nicotine discrimination research. First, these formulations intentionally deliver nicotine to the brain slowly, over minutes (or even hours, with the patch) rather than in seconds as with smoke inhalation [19]. Slower nicotine delivery attenuates or alters subjective effects of nicotine in humans [19, 20]. Second, control over dosing is not particularly good with some NRT formulations [21], including gum or lozenge, owing to variability in chewing or speed of absorption through the buccal mucosa (lining of the mouth). Third, administration via routes other than inhalation, as in smoking, likely influences nicotine discrimination behavior [22], perhaps because of exteroceptive or interoceptive sensory effects

unique to those routes, further limiting generalizability in results between NRT and smoking. One method that overcomes most of these problems is intravenous infusion, which can provide rapid and controlled nicotine doses and can reduce (but not eliminate) peripheral sensory effects [23]. However, intravenous infusion also requires extensive medical monitoring and other practical disadvantages, limiting its use in studies that involve many training and testing sessions.

Faced with the pros and cons of various nicotine delivery methods, we developed a nicotine nasal spray procedure in the mid-1980s to conduct research on acute nicotine effects in humans [24]. (This spray is similar to, but not the same as, Nicotrol®, the pharmaceutical nasal spray product available by prescription for smoking cessation treatment [25]). We used this nasal spray method to begin a program of research on nicotine discrimination in humans in the early 1990s [26], and studies using this spray will be discussed extensively in this chapter. Nasal spray delivers nicotine more quickly than the other NRT formulations, although more slowly than via inhalation, with arterial nicotine levels peaking in about 5 minutes [27]. Unlike the few absolute nicotine doses delivered by NRT products, our approach allows administration of many different doses, as well as correction of doses for body weight to standardize exposure across different subjects. As a result, dosing control is reasonably good [14], and we have found that the dose-response effects of nicotine on cardiovascular and some subjective responses are similar between smoking and nasal spray [28].

The nasal spray method has its own problems, such as sensory irritation in the nose, requiring masking agents to mimic irritation in both active and placebo sprays. In our first nicotine discrimination study, using the nasal spray [26], we were concerned about the degree to which those sensory effects may influence discrimination behavior; subjects could have been distinguishing between spray-administered doses on the basis of their differences in peripheral irritation rather than the interoceptive stimulus effects of the drug. Control over exteroceptive or non-drug-related interoceptive effects inherent in the vehicle for drug delivery is critical for any drug discrimination research. Therefore, we had subjects who demonstrated reliable discrimination behavior try to discriminate the sprays only 10 seconds after administration, which would be soon enough to discriminate the sprays based on any sensory effects but too soon to do so based on the interoceptive effects of nicotine by spray, because of the time required for nicotine to reach the brain. Nearly all were unable to do so, indicating that their discrimination behavior during the formal study was based on nasal spray nicotine's interoceptive effects and not its exteroceptive sensory effects. Thus, overall, the nasal spray method provides a useful tool for the study of nicotine discrimination and other effects in humans. Nevertheless, results presented here may differ if other methods of administering nicotine, such as via inhalation, are used.

2. Overview of Nicotine Discrimination Procedure

Most human drug discrimination procedures are similar, regardless of the drug of interest. Subjects are almost always abstinent from drug for some period of time, to prevent acute tolerance and variable baseline drug levels from altering subsequent responses to

experimenter-administered drug. In our studies of nicotine discrimination in smokers, we require overnight smoking abstinence (at least 12 hours), because of the half-life for nicotine metabolism of 2 hours. Compliance is verified by expired-air carbon monoxide (CO) reading of 10 ppm or less; the half-life for CO is 4 hours. (Our early studies used a cutoff of 13 ppm or less because the older CO monitors tended to overstate CO levels.) Greater duration of abstinence could produce more severe withdrawal symptoms and make compliance much more difficult, leading to a selection bias in the smokers who participate in the research. Recent abstinence from other drugs is also advisable, to prevent the interoceptive effects of those drugs from affecting responses to nicotine, although requiring abstinence from caffeine could introduce caffeine withdrawal symptoms. The details of the procedure specifically employed in our nicotine nasal spray studies of discrimination will be given, but the same general approach is common to most human drug discrimination studies [8, 29]. Subjects are first trained to discriminate a training dose of nicotine (in saline, along with capsaicin and peppermint flavoring to mask sensory effects of nicotine) from placebo (saline plus capsaicin and peppermint) and then tested for acquisition of this discrimination [26, 30]. Most of our studies involve a training dose of 20 μg/kg nicotine, which is roughly comparable to half a cigarette. Subsequent generalization test sessions examine the similarity or difference in responding to a new nicotine dose or another drug, or to the same nicotine doses following pretreatment of some kind, using the training and placebo doses as benchmarks, as described in more detail below. The generalization results are almost always of greater interest, since training is done simply to establish acquisition of discrimination and form the basis for further testing of the effects of various manipulations.

Regardless of the phase of training or testing, different doses are presented in different unmarked spray bottles. (Each dose per trial is presented in eight separate sprays, one per nostril every 20 seconds, to minimize the sensory effects of spray administration. Such a pattern also simulates nicotine intake from intermittent puffing on a cigarette.) Bottles are administered in random order, with trials 20 minutes apart. Therefore, because of the rapid speed of nicotine uptake via spray and the relatively low training dose, we can present multiple trials of training and testing of discrimination in a single session, although acute tolerance can attenuate discrimination on trials later in the session [31]. This approach is done strictly for practical reasons, to minimize subject burden and shorten the number of sessions, and differs from human discrimination of most other drugs, in which typically only one training, testing, or generalization trial is conducted per session [8, 29].

a. Discrimination Training and Testing During training, the two bottles (nicotine training dose versus placebo) are verbally identified by the experimenter with a letter code (spray "A" or "B"), but otherwise subjects are blind to spray contents. Subjects are instructed to relate the interoceptive effects they experience from the spray to the letter code of the particular spray they just received (i.e., "A" or "B") and told that they will later be tested on their ability to tell the sprays apart. During each trial of the test of acquisition, following training trials, subjects able to correctly identify the letter code for the bottle ("A" or "B") that they just received are reinforced by $1

added to their payment for participation. Those who are correct on at least 80% on a minimum of five trials are considered to have acquired reliable discrimination of the nicotine training dose from placebo and continue on in the study. Later sessions usually involve testing the generalization of this discrimination across a range of nicotine doses, often in conjunction with other manipulations such as pretreatment with another drug. Note that we have not tested for the generalization of nicotine discrimination to novel drugs (i.e., substitution) because of practical difficulties in administering those novel drugs by the same nasal spray procedure used to administer the nicotine training doses. However, one intravenous nicotine study not involving formal discrimination procedures has shown generalization of self-report of drug class between nicotine and cocaine or opiates, depending on the nicotine dose [23].

b. Generalization Testing Generalization testing involves a two-choice quantitative procedure, in which subjects place 10 plastic poker chips in either or both of two sides of a box, with one side given the same letter code as the nicotine spray (e.g., "A") and the other side given the same letter code as placebo spray ("B"). Subjects are instructed to place these chips based on whether the spray was "more like spray 'A'" (e.g., the training dose of nicotine) "or like spray 'B'" (the placebo spray). They are told they will receive US $.25 for each "correctly placed" chip, to increase motivation to respond based on the interoceptive stimulus effects they perceive. (In actuality, subjects receive the maximum possible monetary reinforcement at the end of their study participation, since there usually is no "correct" response during generalization.) The percentage of chips (out of 10) placed in the side associated with the nicotine training dose is the measure of nicotine-appropriate responding. A quantal procedure can also be used, in which subjects make a single, dichotomous choice, identifying the spray as like one or the other (i.e., all or none) by circling the letter A or B on a form.

C. BASIC PARAMETERS OF NICOTINE DISCRIMINATION

After our first, preliminary study demonstrating that smokers could discriminate 12 μg/kg nicotine vs placebo via nasal spray, briefly noted previously [26], we sought to establish some of the basic parameters of nicotine discrimination in humans, including whether tolerance might develop to these effects, what the threshold dose for discrimination is, and whether it could be demonstrated that nicotine discrimination in humans was centrally mediated.

1. Chronic Tolerance to Nicotine

We examined whether smoking status influenced sensitivity to nicotine discrimination [32] to see if smokers might become tolerant (i.e., less sensitive), or perhaps even sensitized (more sensitive), to nicotine's interoceptive stimulus effects. (Because of limitations to human research, this test of chronic tolerance involved a cross-

sectional comparison.) We recognized that differences in sensitivity to nicotine due to smoking status could arise from many other possible differences between smokers and nonsmokers. This study and most that followed involved our standard procedure of training subjects to discriminate 20 μg/kg versus 0 via nasal spray on day 1, followed by a test of generalization of this discrimination across a range of intermediate nicotine doses on day 2 (0, 3, 6, 12, 20 μg/kg). Differences in generalization responding across intermediate nicotine doses were of primary interest, as they are in most of our studies. As shown in Figure 15-1, nicotine-appropriate responding during generalization revealed a significant interaction of dose by smoking status, as responding was lower in smokers than nonsmokers at the top dose, 20 μg/kg (i.e., the training dose), but responding was not significantly different at lower doses. Thus, chronic smoking may induce chronic tolerance to the discriminative stimulus effects of moderate or higher doses of nicotine but not to lower doses. If so, these findings could help account for escalation of nicotine intake with chronic use, to overcome tolerance development,

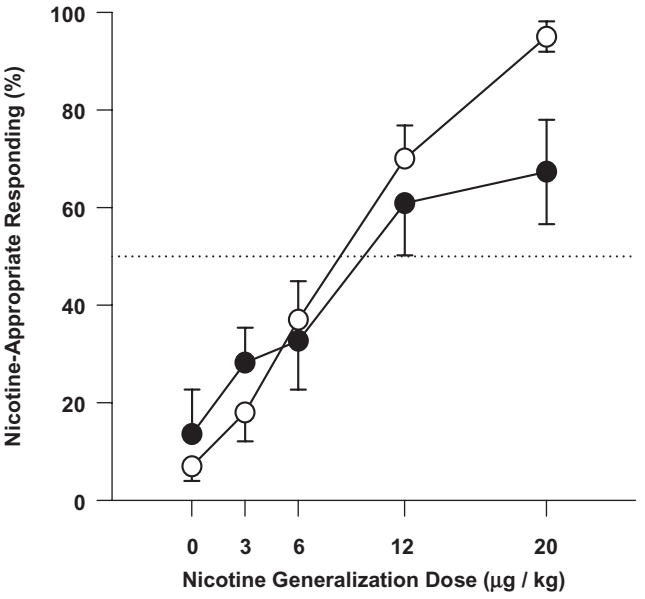

Figure 15-1. Generalization of discrimination across nicotine generalization doses in smokers (filled circles, n = 11) vs. nonsmokers (open circles, n = 10). Differences due to smoking status were observed at 20 μg/kg. Reprinted from Figure 1 in Perkins, K.A., Sanders, M., D'Amico, D., Wilson, A. (1997). Nicotine discrimination and self-administration as a function of smoking status. Psychopharmacology, 131, 361–370. With kind permission from Springer Science and Business Media.

but they also suggest that smokers retain sensitivity to lower nicotine doses. Other research comparing smokers and nonsmokers on nicotine discrimination will be noted later. Because ex-smokers retain much of their tolerance to many of nicotine's effects, despite years of nicotine abstinence [33], comparison of discrimination between current and ex-smokers could determine whether or not tolerance to the discriminative stimulus effects of higher nicotine doses also persists after quitting smoking.

2. Discrimination Threshold Dose

Another parameter of nicotine discrimination that may increase our understanding of the onset and maintenance of tobacco dependence is identification of the lowest dose, or threshold, for nicotine discrimination. With recently passed legislation providing the U.S. Food and Drug Administration with the power to regulate tobacco products, including nicotine levels, establishment of a maximum nicotine content in tobacco cigarettes that is very low could prevent the onset of dependence, as discussed by Benowitz and Henningfield [34]. A great deal of research is needed to identify the threshold dose for nicotine dependence, presumably that dose which initiates reinforcement, but it seems unlikely that a dose that could not be discriminated by nonsmokers would support nicotine reinforcement. Thus, the lowest dose of nicotine that is discriminable from placebo by nonsmokers may provide an initial estimate of the threshold dose for reinforcement. The discrimination threshold in smokers is also useful to know since smokers with access only to extremely low nicotine content cigarettes, below their discrimination threshold, would not be likely to maintain dependence and, therefore, could more easily quit.

To determine discrimination threshold dose, smokers and nonsmokers initially trained to reliably discriminate our standard training dose of nasal spray nicotine (20 μg/kg) from placebo were repeatedly trained and tested on acquisition of discrimination of progressively lower doses of nicotine versus placebo in descending order [35], as in research on human discrimination of other drugs [11]. A second group was trained and tested on discrimination of progressively larger nicotine doses above a very low initial dose (ascending order), to confirm that both procedures would produce the same threshold dose estimate. Discrimination training and testing of only one nicotine dose from placebo occurred on each day. The threshold dose was identified as the lowest dose the subject was able to reliably (80% accuracy) discriminate from placebo on each of two different days, after failing to discriminate the next lowest dose from placebo on two days.

The median threshold dose for discriminating nasal spray nicotine from placebo was surprisingly low and similar between nonsmokers and smokers, 2 μg/kg (approx. 0.14 mg for 70 kg human) and 3 μg/kg (approx. 0.21 mg/70 kg), respectively, although just 1 of 19 nonsmokers, versus 6 of 18 smokers, had thresholds at or above 10 μg/kg, as shown in Figure 15-2. Thresholds were also comparable between the descending and ascending dose order subgoups. The low threshold in nonsmokers suggests that only very modest exposure is needed for drug-naïve individuals (e.g., teens experimenting with tobacco) to perceive nicotine's effects while smoking. The mean blood nicotine

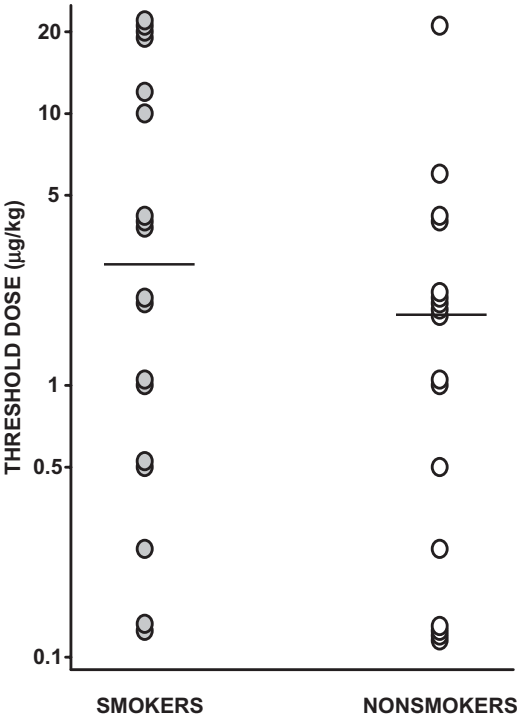

Figure 15-2. Distribution of threshold doses (µg/kg) for nicotine nasal spray discrimination in smokers (n = 18) and nonsmokers (n = 17). Horizontal lines indicate the median of threshold doses for each group, which did not differ. Reprinted from Figure 1 in Perkins, K.A., Fonte, C., Sanders, M., Meeker, J., Wilson, A. (2001). Threshold doses for nicotine discrimination in smokers and nonsmokers. Psychopharmacology, 155, 163–170. With kind permission from Springer Science and Business Media.

level in smokers following intermittent exposure to their threshold dose was less than 3 ng/ml, far below the blood levels seen in smokers even after just one cigarette [27]. Consequently, smokers appear to self-administer doses of nicotine well above their threshold dose for discrimination. Moreover, the more rapid the method of drug administration, such as smoke inhalation, often the stronger the response [19], and so even smaller doses of smoked nicotine may be readily discriminable by smokers and nonsmokers. At the same time, threshold doses varied by over 100-fold within both smokers and nonsmokers, from 0.13 µg/kg up to 20 µg/kg (Figure 15-2), identifying enormous individual differences in sensitivity to the interoceptive stimulus effects of nicotine that are independent of smoking status. Whether this variability is specific to nasal spray administration or generalizes to nicotine via smoking would be important to determine.

3. CNS Mediation of Nicotine Discrimination

A fundamental question is whether nicotine discrimination behavior in humans reflects what we think it reflects, the stimulus effects of the drug in the brain. Despite our efforts to equate all drug administration procedures so that the only difference is the pharmacological effects of the drug, there may be other tell-tale stimulus effects of our drug administration procedure that we overlook. Alternatively, nicotine's peripheral stimulus effects, not central effects, may be what are determining discrimination behavior. Although animal research had shown that central, but not peripheral, nicotinic blockade attenuates nicotine discrimination [36], to our knowledge, there had not been a human study demonstrating central mediation of the discriminative stimulus effects of any drug. Therefore, we examined effects on nicotine discrimination due to pretreatment with mecamylamine, a noncompetitive nicotine antagonist that acts both centrally and peripherally, versus trimethaphan, a fast-acting intravenous peripheral nicotinic antagonist [9]. Attenuation of nicotine discrimination by mecamylamine but not trimethaphan would indicate that the discrimination is centrally-mediated.

We conducted a preliminary study to determine the optimum dose of mecamylamine, prior to the main study comparing mecamylamine versus trimethaphan (versus neither). In both studies, our standard procedure of discrimination training and testing of 0 versus 20 μg/kg nicotine by nasal spray on day 1 was followed by generalization testing across a range of intermediate nicotine doses on subsequent days, under the various pretreatment conditions. In the preliminary study, we tested a range of mecamylamine pretreatment doses (0, 5, 10, 15, 20 mg p.o.) prior to generalization testing. Placebo pretreatment was tested on the first and last generalization session, to assess any effects of time *per se*. All active mecamylamine doses attenuated nicotine discrimination, as shown in Figure 15-3 (collapsed across doses for ease of presentation). Mecamylamine also very clearly attenuated subjective responses to nicotine that may relate to discrimination behavior, including decreased craving and increased "stimulated" and "buzzed" (Figure 15-3). In the main study, nicotine discrimination training on day 1 was followed on three subsequent days by generalization testing of this discrimination after pretreatment with mecamylamine (10 mg p.o.), trimethaphan (10–40 μg/kg/min i.v.; dose was determined by effects on blood pressure), or double placebo, in counter-balanced order. As hypothesized, nicotine discrimination was blunted by the central and peripheral nicotine antagonist mecamylamine but not by the peripheral nicotine antagonist trimethaphan, verifying that the discriminative stimulus effects of nicotine in humans are mediated centrally, as also shown in Figure 15-3.

One implication of this study is that smoking cessation drugs that act centrally on nicotinic receptors may be effective by attenuating the discriminative stimulus effects of nicotine. The newer FDA-approved cessation medication varenicline, a partial agonist of the $\alpha 4\beta 2$ subtype of nicotine receptors, blunts subjective "satisfaction" from smoking in humans [37], presumably due to central nicotinic blockade. Whether it blocks nicotine discrimination in humans remains to be formally demonstrated but, if so, would verify that $\alpha 4\beta 2$ receptors are involved in discrimination, as suggested by rodent research [38], in addition to their known involvement in reinforcement [39].

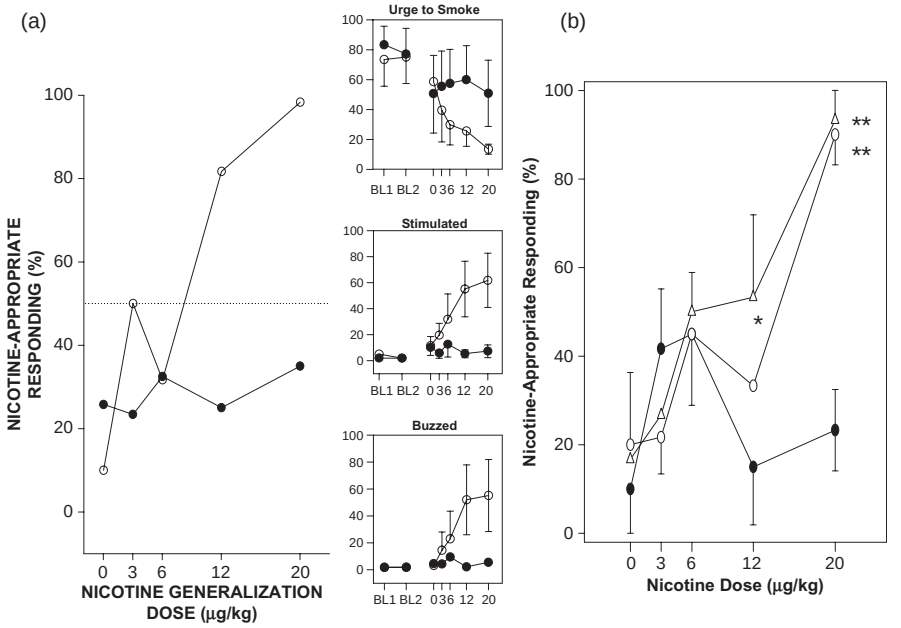

Figure 15-3. a) Mean nicotine-appropriate responding and mean ± SEM subjective responses across nicotine generalization doses averaged for all active mecamylamine pretreatment doses (5–20 mg; filled circles) and for the two placebo pretreatment sessions (0 mg; open circles) in the preliminary study. Subjective ratings at baselines 1 and 2 (BL1, BL2) were obtained at the beginning of each session and just before the first nicotine generalization dose trial (2 hours after pretreatment), respectively. b) Mean ± SEM nicotine-appropriate responding across nicotine generalization doses as a function of pretreatment condition (oral placebo, open circles; 10 mg mecamylamine p.o., filled circles; 10–40 ug/kg/min trimethaphan i.v., open triangles). * $p < .05$, ** $p < .005$ for difference from mecamylamine pretreatment. Reprinted from Figures 1 and 2 in Perkins, K.A., Sanders, M., Fonte, C., Wilson, A.S., White, W., Stiller, R., McNamara, D. (1999). Effects of central and peripheral nicotinic blockade on human nicotine discrimination. Psychopharmacology, 142, 158–164. With kind permission from Springer Science and Business Media.

D. INDIVIDUAL DIFFERENCES AND MODERATORS OF NICOTINE DISCRIMINATION

Despite the establishment of these parameters of nicotine discrimination in humans, discrimination behavior is not an invariant response to drug, dependent solely on the absolute drug dose administered. As with all behavior, nicotine discrimination behavior may vary due to individual differences and to environmental factors, including testing conditions. Few studies have examined individual differences and environmental moderators, perhaps because of the large sample sizes needed to compare responding between groups differing on characteristics and the extensive number of sessions

required to test the influence of various environmental manipulations on discrimination. However, studies of subjective responses to nicotine believed to be related to interoceptive stimulus effects (particularly "feel the effects" and dose "strength") support the likely involvement of several individual difference characteristics in moderating nicotine discrimination. One or two examples of each from the author's research will be described, but many other characteristics may influence discrimination sensitivity.

1. Individual Differences in Nicotine Discrimination

a. Sex Differences In some of our studies of smokers, but not all (e.g., the threshold studies), women tended to be less sensitive than men to nicotine's discriminative stimulus effects. They had more difficulty in acquiring the initial training dose discrimination or showed responding during generalization that tended to be flatter across doses [31, 32]. We have not seen as much of a sex difference in nicotine discrimination among nonsmokers [32], suggesting a sex difference in the chronic effects of nicotine exposure rather than an innate insensitivity to these effects of nicotine in women. (However, we have seen less sensitivity to nicotine reinforcement and reward in women among both nonsmokers and smokers [40].) We have reviewed this research on sex differences in nicotine discrimination in detail elsewhere [30].

b. Genetic Factors Animal research has demonstrated genetic influences on nicotine discrimination [e.g., 41], but no study has examined genetic influences on nicotine discrimination in humans. Yet, some research has identified genetic factors in subjective responses to nicotine that are likely relevant to its discriminative stimulus effects. Ray et al. [42] showed that smokers with the G allele (homozygous or heterozygous) of the mu opioid receptor gene OPRM1 were less sensitive to differences between a nicotine versus denicotinized cigarette in self-reported "strength" and "satisfaction," compared with smokers homozygous for the A allele. We found a few genetic associations with other subjective effects of low-dose nicotine via nasal spray in male nonsmokers, suggesting differences in "innate" sensitivity to the drug [43]. Specifically, greater subjective "feel effects" of nicotine was seen among men, but not women, with the 7 repeat allele of the dopamine D4 receptor (DRD4 VNTR) or the TT versus CT or CC genotypes of the dopamine D2 receptor (DRD2 C957T SNP). Nicotine discrimination as a function of other genes would be an important future research direction. Given the wide variability in discrimination threshold dose within smokers and within nonsmokers (Figure 15-2), genetic factors could be especially relevant to understanding discrimination threshold.

c. Impulsivity Personality factors related to impulsivity are a risk factor for onset of nicotine and other drug dependence [44] and warrant research attention in human studies of nicotine discrimination. In a recent study of nonsmokers, "feel the effects" of nicotine by nasal spray were greater in those with higher scores on a factor reflecting greater delay discounting and probability discounting performance and, in men but not women, those higher in novelty seeking [45]. Importantly, impulsivity may be less important in moderating discrimination among smokers [46], whose general

sensitivity to nicotine (and thus individual variability) is blunted due to chronic tolerance [47].

2. Environmental Moderation of Nicotine Discrimination

In addition to chronic differences in discrimination sensitivity due to these individual difference characteristics, nicotine discrimination varies due to acute environmental factors. Nicotine's interoceptive stimulus effects have neurophysiological correlates [48], but nicotine's subjective, behavioral, and other effects, which may reflect its interoceptive stimulus effects, can be altered by environmental factors [4]. For example, the perceived nicotine content of cigarettes, related to nicotine discrimination via smoking, is altered by expectancy manipulations (i.e., instructions that the cigarette is high or low in nicotine, regardless of its actual content [6]). Moreover, actual nicotine discrimination is a behavioral response that likely can be moderated by the same factors that alter any other behavior. This issue warrants study in its own right but is also important for methodological control in drug discrimination research. Isolating the interoceptive stimulus effects of a drug requires keeping constant the exteroceptive and other, non-drug interoceptive stimuli involved in training and testing. Many environmental factors may alter nicotine discrimination, but only two will be considered—the specific conditions of discrimination training and generalization testing, and concurrent exposure to other drugs.

a. Discrimination Training and Testing Conditions Responding during generalization testing is usually of greater interest than responses during training, but responding during generalization is a clear function of the training conditions, especially the training dose. In two studies, we examined the influence of nicotine training dose on subsequent generalization of responding across a range of nicotine doses. In the first [31], smokers were randomly assigned to a day 1 training dose of either 10 μg/kg or 30 μg/kg via nicotine nasal spray, to learn to discriminate from placebo. All received the same test of generalization on day 2, involving administration of 0, 5, 10, 20, and 30 μg/kg nicotine. Nicotine-appropriate responding was shifted to the left, indicating enhanced discrimination, in the group trained to discriminate 10 μg/kg from placebo, compared to the group trained to discriminate 30 μg/kg from placebo. Our results were very similar to research on nicotine discrimination as a function of training dose in rodents [49].

In the second study [50], we manipulated within-subjects not only the training dose but also the number of response options during generalization testing, to demonstrate how training and generalization testing conditions alter discrimination responding. We also included smoking status as a between-subjects factor (nonsmokers and smokers) to see whether these influences varied due to chronic nicotine exposure. All subjects were first trained to discriminate our standard dose of 20 μg/kg nicotine by nasal spray from placebo on day 1 and then tested for generalization across a range of doses from 0 to 20 μg/kg on day 2. Subsequent sessions were aimed at determining each subject's threshold dose for discrimination, as described previously (see also Figure 15-2). In the last session, that threshold dose and placebo were used as new training doses prior to

repeat assessment of generalization across the same range of nicotine doses, from 0 to 20 μg/kg, as those used in the first test of generalization on day 2. Comparing generalization responding between day 2 and the last day provided a within-subjects comparison of the influence of training dose on responding. We also varied the generalization response options by adding a three-choice procedure to our standard two-choice quantitative procedure, which was described in detail previously. The three-choice, novel-response option can identify stimulus effects of drugs that are qualitatively different (i.e., novel) from the two training doses (i.e., active versus placebo (see [51]; see also Chapter 3). In the three-choice procedure, subjects were instructed to distribute the 10 chips among three bins, labeled A, B, and C, with the first two representing "like spray A" and "like spray B" as in the two-choice procedure, and the last to be used to the extent the spray was "like neither A nor B." The number of chips in the C bin was the measure of "novel-appropriate" responding.

As in our first study of training dose effects [31], we saw a shift to the left in nicotine-appropriate responding of both smokers and nonsmokers when the threshold dose was the training dose, compared to when 20 μg/kg was the training dose. Mean threshold doses were similar between smokers and nonsmokers (3.5 and 1.9 μg/kg, respectively). There was no effect of smoking status on generalization responding with the two-choice procedure. However, under the three-choice procedure, nonsmokers emitted more nicotine-appropriate responding at low generalization doses and more novel-appropriate responding at higher generalization doses when the threshold dose was the training dose than when 20 μg/kg was the training dose.

b. Concurrent Drug Use The stimulus effects of different drugs may combine in additive or complex ways when they are used together, a common occurrence given the high prevalence of drug use among smokers [52]. The discriminative stimulus effects of nicotine in combination with other drugs has been thoroughly examined in rodents [36] but not in humans. However, we have conducted separate studies examining nicotine discrimination following pretreatment with alcohol or caffeine, and those studies will be briefly presented. An influence of alcohol or caffeine consumption on nicotine discrimination was of interest to us because it could potentially help explain why alcohol or caffeine may increase smoking behavior [53, 54].

In our study of alcohol pre-treatment effects on nicotine discrimination [55], smokers were trained to discriminate 20 μg/kg from placebo on day 1 and then tested for generalization across a range of nicotine spray doses on three subsequent days, following pretreatment with placebo, 0.4 g/kg, or 0.8 g/kg alcohol, with one alcohol pretreatment condition per session. No effect of alcohol pretreatment was seen on nicotine discrimination behavior, consistent with rodent research [56] and indicating that alcohol's influence on smoking behavior is not likely to be due to changes in nicotine's discriminative stimulus effects.

Caffeine pretreatment effects on nicotine discrimination were examined using very similar procedures, in which smokers were pretreated with 0, 2.5, or 5.0 mg/kg caffeine p.o. before each of three generalization testing sessions, following day 1 training of discrimination between 20 μg/kg and placebo [57]. Identical to the alcohol pretreatment study, caffeine pretreatment did not affect nicotine discrimination behavior. These find-

ings are partly consistent with a prior human study [58], which found that pretreatment with a small caffeine dose, 50 mg (about 0.7 mg/kg), had no effect on discrimination of the active nicotine generalization doses of 0.25, 0.5, or 1.0 mg via gum. Yet, Duka et al. [58] did find that caffeine pretreatment increased nicotine-appropriate responding to placebo gum, suggesting partial generalization between caffeine and nicotine (i.e., interoceptive stimulus effects of low-dose caffeine were similar to those of nicotine). Overall, however, concurrent caffeine intake appears to have little influence on nicotine discrimination.

Although we found no influence of alcohol or caffeine on nicotine discrimination, many other environmental factors may moderate nicotine discrimination. Some of these are intrinsic to nicotine consumption by smokers in the natural environment but remain virtually ignored by researchers. For example, exteroceptive and other interoceptive stimuli that commonly accompany nicotine via cigarette smoke, such as the sight, smell, and taste of tobacco, influence subjective and behavioral responses to smoking [59] and so may influence discrimination behavior. As noted at the start of this chapter, such influences must also be controlled in research examining discrimination of nicotine per se. Similarly, expectancies about the nicotine content of cigarettes can alter subjective effects related to nicotine discrimination (e.g., self-reported amount of nicotine intake), often more than the actual nicotine content of the cigarettes alters those effects [60; see also 6], and also should be controlled during discrimination research. Other environmental factors that influence smoking reward or reinforcement, such as negative mood [60], may also moderate nicotine discrimination.

E. CONCLUSIONS

For practical reasons, most nicotine discrimination research involves drug administration via novel means, and most of our research involved nicotine nasal spray. How these results generalize to discrimination of nicotine administered via cigarette smoking is not clear, limiting the conclusions that can be reached. However, the interoceptive stimulus effects of nicotine per se are discriminable by humans and are centrally mediated. These findings suggest that human nicotine discrimination may be a useful screening tool for smoking cessation medication development [e.g., 61], such as by indicating whether a novel compound acts at nicotine's central site of action. Nicotine discrimination threshold dose is similar between smokers and nonsmokers but varies widely between individuals. Yet, the threshold for most people is far below the nicotine content of almost all cigarette brands. That observation may have important implications for FDA regulation of nicotine in cigarettes, if the goal is to reduce the nicotine content below a level that will sustain dependence [34]. Individual differences in nicotine discrimination are understudied, but nonsmokers and men may be more sensitive to the discriminative stimulus effects of moderate nicotine doses. Genetic factors and impulsivity warrant more attention as potential influences on nicotine discrimination. In all this research, training and testing conditions must be carefully controlled since they strongly affect nicotine discrimination behavior. Other environmental moderators of discrimination are not as clear, but concurrent use of alcohol or caffeine, drugs very

commonly consumed by smokers, does not appear to alter nicotine discrimination. Factors known to influence subjective and reinforcing effects of nicotine, such as nicotine dose expectancies, negative mood, and the presence of smoking cues, should be examined as moderators of nicotine discrimination and controlled in other discrimination studies.

Acknowledgments The author's research described here and preparation of this chapter were supported by NIDA grants DA08578, DA19478, and DA027449. The author thanks Carolyn Fonte and Annette S. Wilson, along with many other past assistants, for their help during the conduct of these studies.

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16

DRUG DISCRIMINATION: A PERSPECTIVE

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For me, drug discrimination (DD) over the past decades has been an object of scientific interest in its own right; an investigational tool in studies of receptors, cellular and "system" mechanisms of drug action; a source of inspiration and of, at times, radically new concepts concerning neurobiological processes and pathophysiological mechanisms; and the starting point of several, thankfully, successful drug discovery projects. Here, I will point to some of the issues that have particularly impressed me and that are not considered elsewhere in this volume and perhaps less than sufficiently

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

so in other literature. As per the editors' request, many of those issues concern my own laboratory's work, probably because it is what I know best or have been especially intrigued by. Before going into those issues, I feel I should indicate that it all began with the insidious development of a both technological and theoretical divorce—and a revolution of sorts—that some might argue remains to be settled today.

A. STATE DEPENDENCE AND DRUG DISCRIMINATION

Due in large part to Dr. D. Overton, the 1950s–1960s witnessed a huge surge in research on drug-produced state dependence (StD). The original and basic observation being made in an StD experiment is that a response that has been learned in a given drug "state" is better remembered when the subject finds itself again in that state as opposed to the normal state. Thus, the paradigm is one where one response is learned in one state and where that response's retrieval (or, in operational terms: its performance) is tested for in the same or another state. However, in the 1970s, designs were also devised where in the same subject one response was trained after one pharmacological treatment and another response after another treatment. In one such design that we implemented, rats were first trained, in the normal state, to press two different levers for food according to a fixed ratio 10 (FR 10) schedule; thereafter, the rats were trained to press one lever after injections of saline and another lever after injections of the opiate, fentanyl. Importantly, or so it seemed to me, the rats remembered the two levers and the FR 10 schedule after the first administration of either pharmacological treatment. The second stage of training acted to make the rats learn to express the perceptual discrimination that they seemed to make between the "discriminative stimulus" (DS) effects of the two treatments much in the same way as subjects can discriminate, say, a green from a red traffic light, and execute a differential operant response as a consequence thereof. I considered this to constitute a model of the subjective effects of drugs in man and to no longer concern memory's StD [1, 2]. Be that as it may, in fact, researchers began to abandon the StD paradigm; to implement what is now known as the DD technology; to refer to DS rather than StD properties of drugs; and to denote those as "cues" rather than "states." Ever since, I have rather enthusiastically worked with the DD paradigm, but am puzzled by the scientific community's disengagement from StD research and its deafening silence about what I see as a true paradigm shift and its implications. Not that this has refrained me to conduct—in almost complete but deliciously splendid solitude—StD research as well and uncover some of the marvels of memory's StD [3].

B. DRUG DISCRIMINATION IN RECEPTOR PHARMACOLOGY

It soon became apparent that the DD paradigm offers an exquisitely specific, selective, and sensitive approach to the in vivo analysis of drug-receptor interactions (see also Chapter 1). This was especially the case with opiate receptors for which sophisticated ligands and considerable background knowledge were available; the issue has been well covered elsewhere. The following, though, is of particular note.

Molecular pharmacology at the time theorized that the response to drug-receptor interaction depends on the ligand's affinity and efficacy (intrinsic activity) at the receptor. However, a DD analysis of opiate ligands exerting different efficacies revealed that the magnitude of receptor activation determines not just the quantitative magnitude (e.g., discriminability) of the DS effects, but also their quality [4]. Ligands exerting different levels of intrinsic activity produce different discriminative responses in much the same way as quantitatively different wavelengths of light generate effects that vary qualitatively as colors; a particular quality of response to receptor activation occurs within a limited, perhaps narrow bandwidth of activation, but not with either lower or higher levels of activation. It was only later that molecular pharmacology uncovered that, at G protein–coupled receptors such as the μ-opiate receptor, G proteins can be engaged to different extents through a single receptor depending on the agonist (i.e., agonist-directed trafficking of the receptor stimulus [5]; this illustrates how the in vivo and thus low-resolution, high-integration DD paradigm can anticipate the science derived from high-resolution, low-integration in vitro technology.

This interesting insight also proved useful. A similar DD analysis of LSD's effects suggested that they result from a low-level bandwidth of 5-HT (perhaps 5-HT_2-type) and DA (perhaps D_2-type) receptor activations [6]. LSD constitutes a drug model of psychotic pathology, and we found that existing putative 5-HT receptor antagonists produced both partial LSD-like and LSD-antagonist effects. This made us set out to identify agents that would fully block LSD's discriminative effects and not act as partial LSD-like agonists. Thus was discovered risperidone [7], a most successful antipsychotic that in 2007 also became the first FDA-approved treatment of pediatric schizophrenia.

C. DRUG DISCRIMINATION AND SUBJECTIVE DRUG EFFECTS

The notion that DS effects in animals may be homologous to subjective drug effects in man similarly proved useful. Opiates in man produce subjective effects that are involved in opiate abuse . . . and also slow gastrointestinal motility. We found that, in rats, various opiates produce both opiate-DS and motility-impairing effects, that the potencies with which they exert these two effects do not correlate [1] and, consequently, set out to identify an opiate that would impair motility without exerting (fentanyl-like) DS effects. Thus was discovered loperamide [8], which at some point became the mainstay treatment of diarrhea and saved scores of patients from at times fatal dehydration and ion loss and is otherwise of traveler fame; loperamide produces no opiate-subjective effects in man and is not being abused.

Dr. A. Weissman took DD's capability of assessing subjective effects a most exciting step forward. By definition and *par excellence*, pain is a subjective experience. We, at the time, were establishing the rat with adjuvant arthritis as a chronic pain model and eventually succeeded in demonstrating and quantifying such pain but did so using measures that, however innovative, fell short of accessing pain's true nature [9]. Weissman [10] demonstrated that, unlike healthy controls, arthritic rats can discriminate aspirin from saline, suggesting that the rats were actually discriminating the subjective

experience of pain from its (relative) absence. To my knowledge, this arguably constitutes the first-ever observation in animals of pain as the latter is defined essentially.

Another highly innovative DD model of subjective pathology involved rats that were rendered opiate-dependent by chronic morphine administration. Dependent rats were found to readily discriminate opiate antagonists, and pharmacological analysis of stimulus generalization in these animals suggests that they specifically discriminate the centrally originating, subjective experience of opiate withdrawal [11]. With different strands of DD research coming full circle, morphine but not loperamide was found capable of reversing the DS effects of antagonist-induced withdrawal.

D. NEW CONCEPTS OF OPIATE TOLERANCE, SIGNAL PROCESSING, PAIN, AND ANALGESIA

In the 1970s, it was considered that tolerance develops to opiates, so much so that such tolerance was held as a defining feature of opiate action; with different opiate actions, tolerance might develop at differential rates, but the phenomenon was thought to be intrinsic to the interaction of a μ opiate agonist with its receptor.We trained rats to discriminate fentanyl from saline and determined the fentanyl generalization gradient several times over a 4-month period during which fentanyl continued to be repeatedly injected. Though the animals demonstrated definite tolerance to fentanyl analgesia, the animals' sensitivity to the compound's DS effects remained unchanged and we concluded that tolerance does not develop to opiate DD [12]. (Vivid controversy ensued for two decades, but a 1995 review, I believe, may have portrayed the issue adequately [13].) But how is one to imagine mechanisms whereby one but not another effect of opiate receptor activation demonstrates tolerance when—as all evidence indicated—the two effects are mediated by the same CNS receptors? In pondering this question, three notions came to mind. One was that of a neuron's refractory period, that is, of the time period following excitation during which the neuron is not or is less excitable. Another notion, leaky integration, came from electrical circuit theory. It signifies that the integrator's current output is entirely created by past input and is leaky; in simple numerical terms, it is an average of past input whereby the weight of that input is somehow attenuated. Finally, the use of radar technology in WWII had prompted the advent of Signal Detection Theory (SDT) that was soon applied also to biological processes and, actually, to DD [14, 15]. SDT establishes the frequency distribution of the dots that at any time appear on the radar screen and sets an arbitrary criterion (e.g., that which separates the 5% highest-intensity dots from the remaining 95% of the population). SDT then considers those 5% dots to be "signals," the other 95% being "noise." Note that SDT arrives at an all-or-none decision (a nominal, or quantum variable) about inputs that vary in a graded manner and implicitly does so by comparing an individual dot's intensity with that of the current population.

I collapsed these notions in an effort to imagine how pain and opiate analgesia come about. Consider a neuron whose firing rate depends on the physical stimulation of receptors and the question as to how this input to the CNS can be processed so as to generate a pain sensation (the receptors being nociceptors as well as opiate recep-

tors that act to increase and decrease firing rate, respectively). The processor continuously receives this (graded) firing rate as input and, actually, as its only input. Unlike the case with radar, the processor thus has no simultaneous information ("noise") available with which to compare the current input and therefore generates its own comparator. This it does by computing the moving average of past input; it does so over a limited period of past time (what I called the "sample period") and averages the past input in a leaky manner (by attributing a full weight to recent input, but progressively less weight to the input that is progressively more remote in time). The processor, then, continuously determines the difference between the current input and the current average. This difference varies in a graded fashion over time and can be exploited to assess the graded intensity of the physical stimulation; by setting a criterion value in a SDT-like manner, it can also be exploited to generate a quantum outcome.

Wonderfully, this theory offers what I was looking for, that is, a concept of how opiate analgesia but not opiate DD demonstrates tolerance in spite of both being mediated by the same opiate receptors [13, 14, 16].[1] Assuming that the primary receptor action of opiates is immutable (that no tolerance develops to that action), the DS effect, which to me is a quantum outcome, will obviously never change, the criterion in question being set by the training dose. (The experimental manoeuvres that purportedly demonstrate tolerance to opiate DD, so I argue, act to reset the criterion [13].) At the same time, and while continuing to assume that no tolerance develops to the opiate receptor action, the theory predicts that prior opiate exposure will nonetheless act to diminish the (graded) analgesic effect (i.e., to induce a tolerance that I therefore call "apparent").[2]

The theory implies that signal processing in pain-processing systems is bidirectional, or paradoxical. That is, any stimulation will produce a "1st order" effect (that is ipsi-directional to the stimulation, i.e., positive and negative with the stimulation of nociceptors and of opiate receptors, respectively), but also a "2nd order" effect that is opposite in sign to the 1st order effect. Thus, nociceptive stimulation induces pain

[1]The 1995 paper [13] capped two decades of at times ferocious controversy with regard to whether tolerance develops to opiate DD, but the same debate has not so far been considered with regard to the discrimination of other drug classes. [Also missing in the literature are DD studies that examine the tolerance issue with a drug that is known to cause tolerance by identified pharmacological mechanisms (e.g., a compound that induces the enzyme that metabolizes the compound to inert molecules).] Such was the force of the conviction that tolerance develops to opiates that *Pharmacological Reviews* accepted the 1995 paper in the understanding that I submit another manuscript explaining how the absence of tolerance to opiate DD could be reconciled with entrenched dogma that tolerance develops to opiate analgesia. *Pharmacological Reviews*' editor accepted that (1996) paper [16], too, but not without introducing a new disposition concerning the publication of controversial reviews on that occasion [37].

[2]That the opiate receptor action remains immutable in spite of there being apparent tolerance would seem to also follow from the following observation that is widely reported with isolated tissues or whole organisms. Continuous exposure to an opiate induces an apparent tolerance that eventually reaches a particular asymptote. At that point, the preparation also has become dependent; treatment discontinuation or the addition of an antagonist elicits withdrawal. But no withdrawal occurs when exposure is continued, indicating that the opiate continues to suppress withdrawal, which it does by a maintained, unchanged capability to activate the opiate receptor.

followed by refractoriness (hypo-algesia) whereas opiate receptor activation causes analgesia followed by hyper-algesia (and allodynia or, frankly, pain). Repeated or continuous stimulation acts to make the 2nd order effect grow and neutralize the 1st order effect. While the theory so far had only been an *a posteriori* account of existing findings, it now offered the *de novo* prediction that the apparent tolerance that develops to opiate analgesia should not develop in as much as the subject is co-exposed to matching nociceptive stimulation. Taking my breath away, experiments confirmed this prediction [17] and encouraged me to undertake what I guess has been one of my boldest, longest research efforts. Indeed, the theory says that nociceptive stimulation induces 1st order pain followed by 2nd order hypo-algesia, and that such continuous stimulation should act to make the 2nd order analgesia grow. I thought that there might perhaps exist some mechanism that would mimic the CNS effects of peripheral nociceptive stimulation and in so doing generate effects that would constitute the mirror inverse of opiate actions. In particular, such a mechanism might produce some initial hyper-algesia but would also induce an analgesia that would grow rather than decay (i.e., by "inverse apparent tolerance") and present an unprecedented treatment of chronic pain even in opiate-tolerant subjects. Again taking my breath away, such is precisely what experimental evidence proved to be the case with very-high-efficacy 5-HT_{1A} receptor activation, a molecular mechanism that challenges the primacy of the millennia-old opiates in the treatment of pain [18, 19].

Even as opiates induce a 2nd order, insidiously growing pain, the 1st order analgesia that they also produce can offer powerful relief of such pain. A relief that is particularly reinforcing, albeit temporarily so and is often followed by an aggravation of opiate pain. I thus have come to view opiate addiction as self-medication of opiate pain; note that this view allows but does not require that opiates produce an intrinsic rewarding action [16, 20]. Of further interest, all drugs of abuse have in animal studies been shown to produce analgesia in some experimental conditions. They, therefore, may perhaps all also induce some form of paradoxical pain that in a similar manner gives rise to self-medication.

E. DRUG DISCRIMINATION: AN ELEMENTARY PARTICLE OF BEHAVIOR AND MORE

From the outset it seemed clear to me that the StD paradigm is about the ability to—not necessarily consciously—remember while being in a particular state. That is, to remember to a graded extent, the dependent variable being measured by scales that are ordinal or can even be of interval nature. In contrast, the DD paradigm is about perceptual (interoceptive) discrimination and a decisional process that is an attribute of consciousness and results in a response that constitutes a nominal variable; in a drug–saline discrimination, the drug-appropriate response constitutes a quantum, an elementary particle of behavior. However, most DD researchers at least implicitly consider DD's dependent variable to be graded, expressing the data in terms of the percentage of drug-appropriate responding rather than in terms of the percentage of subjects selecting the drug response. We agree to disagree on this issue (e.g., [21,

22]), an issue that may be no easier to resolve than the difficulty which physics has with formulating a "theory of everything," one that encompasses the current realms of both quantum mechanics and the theory of relativity. Be that as it may, following are some of the excitements that we have encountered from the quantum perspective as it applies to the discrete-trial, two-lever, FR 10, food-reinforced procedure that we implemented throughout:

- The quantum perspective appears pivotal in identifying the nature of partial drug-appropriate responding. In experiments analyzing agonist and antagonist effects of various opiates [4, 23], such responding does not signify that the test drug resembles the training drug partially but rather that only part of the subjects generalize, whereas the other do not. It further signifies that different subjects discriminate different qualities of the training drug's DS properties and that that quality in turn depends on the magnitude of receptor activation that the training dose induces in the subject.
- The quantum perspective allows the determination of the lowest generalized dose (LGD) in individual subjects, to identify the characteristics of the LGD's frequency distribution, and to reveal that over time the LGD actually oscillates [24]. The mechanisms and biological significance of this oscillation remain to be explored.
- On occasions, the graded perspective definitely leads to erroneous conclusions that the quantum approach avoids. In rats trained to discriminate fentanyl from saline, haloperidol reliably induces a substantial percentage of drug-appropriate responding, an outcome that leads proponents of the graded perspective to infer that haloperidol partially generalizes with fentanyl. Quantum analysis of the same data indicates that not even some (part) of the subjects generalize haloperidol [24].
- Other than its measure of stimulus generalization, the graded perspective records the total amount of responding. The quantum approach offers more. Additionally, in our procedure, it allows one to measure latency and accuracy ("FRF") of the discriminative response; to realize from these measures that the animals hesitate ("doubt") about their lever choice at doses close to the LGD; to realize that the behaviour effectively reflects a decision rather than StD memory; to derive the percentage of the drug-appropriate responding that occurs after the discriminative response has been executed; and to infer that in the DD paradigm, responding is governed by an acquired win-stay/lose-shift rule [24, 25].
- The five dependent variables in our procedure[3] can be exploited to investigate issues other than the DS properties of drugs; following are two examples.

[3]In this discrete-trial, two-lever, FR 10, food-reinforced procedure the recorded data and dependent variables that we used have typically been the lever choice, its latency and the number of responses made on either lever before presentation of the first reinforcement (FRF), the total number of responses, and the percentage of those responses that were made on one of the two levers. Other (discrete-trial!) procedures are of course possible, data could be recorded in more detail, and further variables of interest could be derived there from.

- The injection of haloperidol in fentanyl-saline trained rats does not produce stimulus generalization but induces a significant number of drug-lever responses after the animals have selected the saline lever "perfectly" at the beginning of the session. This appears to imply that haloperidol, at appropriate doses, interferes not at all with the secondary motivation that drives the food-reinforced lever choice nor with the acquired win-stay/lose-shift rule, but blocks the primary reinforcing action of the food that is delivered after the discriminative response has been executed. Thus, like saline-tested but non-rewarded controls, the haloperidol-tested rat, after its saline lever selection, behaves as if no reward was delivered (as if it has "lost") and, apparently remembering and implementing the acquired win-stay/lose-shift rule, shifts responding to the drug lever (hence the wording "win-shift"). Other established antipsychotics exert this effect to an extent that is more limited and with less behavioural specificity than haloperidol. Haloperidol's action profile here is of interest in view of its peculiar profile of clinical ("incisive") action in schizophrenia. This in turn has eventually led us to the discovery of F15063, an agent that induces haloperidol-like win-shift but no haloperidol-like catalepsy [24].
- Without any cynicism whatsoever, we also have used this DD procedure to investigate scopolamine-induced StD of memory. Scopolamine constitutes a drug model of the disordered memory that is associated with Alzheimer's disease and other pathological conditions (e.g., medial temporal lobe lesions). Alzheimer's patients demonstrate temporally graded retrograde amnesia, an amnesia for past events where the memory loss for recent events is more pronounced than for the distant past in the patient's ontogeny. Much to our surprise, scopolamine did not interfere with a rat's ability to learn FR 10 lever pressing for food, or not more so than a peripheral muscarinic acetylcholine antagonist. Instead, scopolamine robustly induces both drug-to-saline and saline-to-drug StD.

Fentanyl-saline discrimination in our procedure required some 35 daily training sessions or, at 5 sessions/week, some 7 weeks, and it and the other parameters continued to evolve for, in all, several months, after which the parameters stabilized and daily performance of the DD task could be monitored for an even longer time, eventually generating data on learned behaviors that spanned a large part of the rat's ontogeny. Thus, the procedure can be used to probe the retrieval for the learning (i.e., for the various changes that over time occur with the different behavioural parameters) that occurs at different points in the course of ontogeny. We found that acute scopolamine injections made the animals behave the way they did at some time in the past, demonstrating retrograde amnesia. The amnesia appeared to be temporally graded; the higher the dose, the more the test behavior resembled that of the more distant past. This offers, an animal model (perhaps the fish) of temporally graded retrograde amnesia; the research further suggests that, more so than its mere decrease, the lability of cholinergic tone (and of the associated mnemonic state) may constitute a chief cause of Alzheimer's-like disordered memory [26].

F. WHEN DEPENDENT VARIABLES CHOSE THEIR PHARMACOLOGY

Even where generally the results of DD experiments are remarkably coherent with the training drug's pharmacology, as is the case with fentanyl, I have been surprised by the versatility of such results under different conditions. Like many of us, among the outcomes that we have used to investigate the pharmacological features and mechanisms underlying fentanyl's DS effects are the following: the shape and slope of the fentanyl generalisation gradient and its ED_{50}; those of, say, morphine in generalizing with fentanyl; those too of, say, naloxone in antagonizing the stimulus effects of fentanyl; the extent to which opiates with different efficacy and nonopiates generalize with and/or antagonize fentanyl as well as the formal features by which they exert those effects. These outcomes vary and depend on—among other conditions—the fentanyl training dose, the magnitude of that training dose relative to the rat's lowest discriminable fentanyl dose, whether the discriminandum is of the drug-saline or drug-drug type, and on the symmetry/asymmetry of reinforcement in a DD procedure where the discriminative response is also an instrumental one [27–32].

I believe we understand much of this variation in opiate DD outcomes from the vantage point of the agents' efficacy at the (μ) opiate receptor. But it is logical, in addition to straightforward knowledge of receptor pharmacology, that I need to understand why. Fentanyl's ED_{50} in generalizing with the 0.04 mg/kg training dose is reliably lower than 0.02 mg/kg in rats discriminating 0.04 mg/kg from saline, the ED_{50} in rats discriminating 0.04 mg/kg from 0.02 mg/kg is—"must of course be"—somewhere between the two training doses. Worse, displaying my ignorance for all to see, I naively use intuitions about "attention" and "response bias" and vaguely descriptive language such as "pharmacologically unspecific" when it comes to findings that at fentanyl training doses that are or are near to the lowest-discriminable dose, nonopiates generalize to fentanyl, or when the food reinforcement conditions are asymmetrical and the fentanyl gradient varies as a consequence thereof. It seems to me that the vantage point of the agents' efficacy at the opiate receptor remains useful in explaining some of those variations, but then, on the assumption that even with the same 0.04 mg/kg fentanyl training dose, the independent variables can somehow determine the level of intrinsic activity that the outcome variables implement. This, as noted above, appears to happen when noncontingent drug administrations change the gradient such as to suggest that tolerance develops to opiate DD [13]. But just how these variations come about remains a wide-open, exciting area for future research.

G. TWO FURTHER MYSTERIES

If one adheres to the quantum analysis of DD data, then the evidence on the 0.04 mg/kg fentanyl versus saline discrimination in our habitual procedure indicates that an adequate, fairly high bandwidth of μ-opiate receptor activation is required to produce stimulus generalization; nothing else will do and all else will induce saline-appropriate responding . . . which from an operational perspective also denotes a generalization

with saline. The saline DS here thus is one that accommodates not just saline, but also any drug that is nonetheless discriminable; we know the DS effects of different drug classes to be different, and I thought it appropriate to refer to the saline DS here as a multidimensional "saline space" [33]. But what with training rats to discriminate from saline either one or another of two well-discriminable drugs (here: 0.04 mg/kg of fentanyl or 10 mg/kg of cocaine)? It turns out that such discrimination is feasible [34]. This "OR" discriminandum can be used for different purposes. What I wonder about particularly is whether by extending the ORs—by implementing not just two but a multitude of discriminable OR training drugs—one can perhaps arrive at experimentally grasping the "saline silence," the point where presumably no drug-produced interoceptive effect intrudes. That silence should be interesting, and one that therapeutic agents may wish to achieve.

Using our DD procedure, I have been puzzled by cocaine. We train the rats to perform the FR 10 lever-press ratio for food and implement the drug versus saline discriminandum once the rats adequately execute the schedule on both of the two levers. At this point, 10 mg/kg of cocaine is a drug discriminator's dream; the cocaine does not to any noteworthy extent hamper the operant responding and the rats acquire the discrimination with a sessions-to-criterion that is among the fastest that we have encountered. Of note, there is considerable "bias" here: the rats are significantly faster in learning to respond drug after cocaine than they are in responding saline after saline; after having reached the discriminative training criterion, the rats make mostly commission rather than omission errors; and the cocaine gradient displays an ED_{50} that is exceptionally lower than the training dose [35, 36].

But in attempting to study cocaine StD, I was astonished to find that with a 10-fold lower cocaine dose, rats could not at all be trained *de novo* to acquire to (FR 10) lever press for food (unpublished data). Can anyone please explain?

H. EPILOGUE

My DD experience now has been one of three decades. Those years were of an unprecedented reductionism, what with the advent of in vitro receptor binding, molecular biology, biological psychiatry, the genome, molecular design, combinatorial chemistry, high-throughput screening, virtual screening, information technology and the evolving landscape of scientific communication. Yet more than many other technologies and research tools, DD to me has been an exceptionally prolific source of data, of insight and inspiration, of novel concepts. Why? One major reason certainly is that DD is not reductionist; instead, it is an indispensable complement to high-resolution technologies and the reasoning that can evolve there from. DD being an in vivo technology, its analyses, while of low resolution, evolve at a high level of integration, one where system properties emerge ("emergent properties") that do not appear at lower levels of integration. More so than many other in vivo technologies, DD offers exquisite specificity and refinement in the experimental analysis of drug action. And uniquely among in vivo technologies, DD accesses the realm of subjective, interoceptive perception; it asks the question "What do you feel?" and provides the answer to the highest of scientific standards.

What has impressed me particularly is DD's contribution to concepts for and the actual realization of drug discovery, at least for as far as our areas of inquiry has been concerned. One can more or less readily conduct studies, generate data, obtain insights, propose new theories, and publish, which is what public research is mostly and thankfully about. But the goal to make advancements in the treatment of disease forces one to bring whatever hypothesis that one has to a particularly hard, unforgiving test; to an exceptional, at times ultimate, level of validation. If a technology can bring one to that point, then something may have been seriously right about it.

However, this theory does not stand quite alone. "Counterirritation" has been known for a long time; the superficial irritation (e.g., induction of inflammation) of the skin that paradoxically relieves pain arising from deeper or adjacent structures. Prior to my 1978 paper, Dr. R. Solomon (for review, see: [38]) proposed a psychological, "opponent process" theory of opiate motivational processes that has some important formal features in common with mine and which I became aware of only in the late 1990s. Also in an independent manner, Dr. R. Bond [39] proposes paradoxical drug effects ("paradoxical pharmacology"; specifically: cardiac and pulmonary actions of β-adrenergic antagonists) that bear much resemblance to the same formal features.

Acknowledgments I am profoundly grateful to the stimulating and enchanting community of DD scientists, brothers-in-arms and sparring partners both. While I visited many another area and technology, opiate DD to me has been a defining training ground; I owe much to Drs. C. France, S. Holtzman, W. Koek, E. Walker, J. Woods, and A. Young. And to Dr. P. Janssen, who viewed DD as the "first true technique of experimental psychopharmacology" and allowed me boundless intellectual liberty [7].

Sadly, Dr. Francis Colpaert passed away during the publishing process of this book. This chapter is his final publication.

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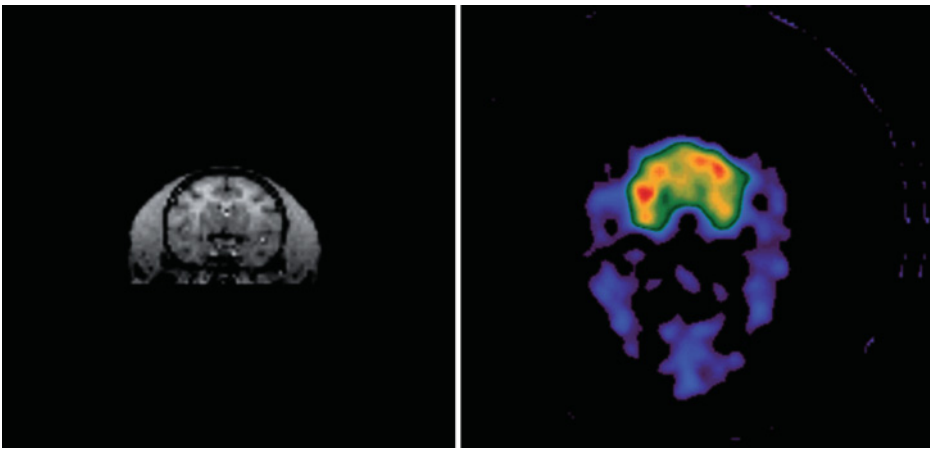
Prefixes for chemical substances, be they position-numbers, Greek symbols, or heteroatoms, are ignored in the alphabetical listing that follows. For example, 5-HT and 4-hydroxyamphetamine both are listed under "h", as is *N*-hydroxyamphetamine. Stereochemical indicators (i.e., *cis*-, *trans*-, *d*-, D-, *l*-, L-, *S*-, *R*-, *E*-, Z-, and ±) also are ignored (e.g., *Z-isomer* is listed under "i"). Rodents, particularly rats, are the most commonly employed animal species in drug discrimination studies (*see* Table 2-1); hence, there are no entries below for drug studies specifically indicating where "mice" or "rats" were used. However, studies with other animal species are identified. Finally, certain terminologies relating to drug discrimination studies are commonly employed by most investigators and are frequently used throughout this book. Consequently, these oft-used terms typically have only one or two subject entries, and the entries refer to their definition or method of application (e.g., *substitution*); each individual use of these terms in this book is not specifically cited. Also note: Page numbers in *italics* refer to figures, whereas page numbers in **bold** refer to tables.

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



MRI SPECT

Figure 7-5. Results of a typical experiment where [^{123}I]DOI was injected into a Rhesus monkey and imaged by SPECT as described in the text [12]. The image was taken 4h post-injection and indicates accumulation of specific [^{123}I]DOI binding in the cortical region of the brain. MRI indicated the same brain region of the coronal SPECT viewed through the basal ganglia. (Unpublished photo courtesy of Dr. Kan Sam Lee, NIH.)