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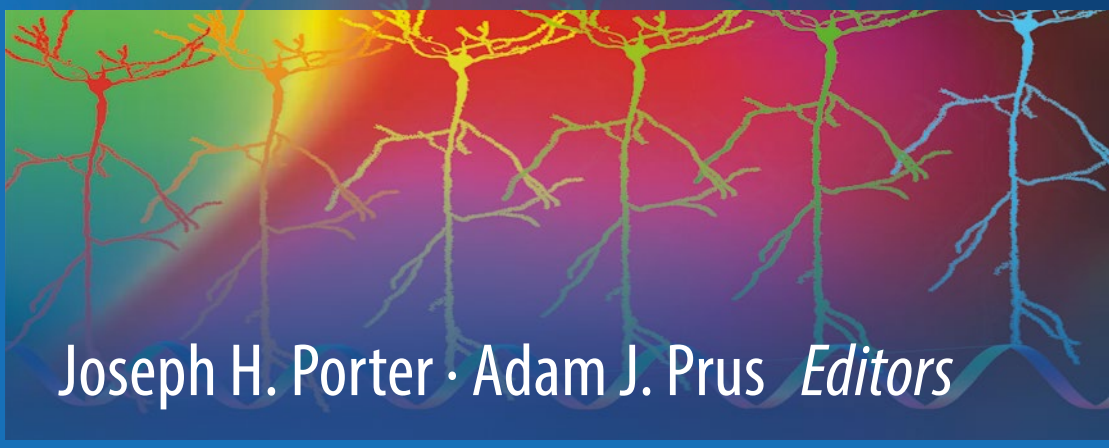

Joseph H. Porter · Adam J. Prus *Editors*

The Behavioral Neuroscience of Drug Discrimination

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Joseph H. Porter • Adam J. Prus
Editors

The Behavioral Neuroscience of Drug Discrimination

Springer

Editors
Joseph H. Porter
Department of Psychology
Virginia Commonwealth University
Richmond, VA
USA

Adam J. Prus
Department of Psychological Science
Northern Michigan University
Marquette, MI
USA

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Preface

The year 2018 marks the 40th anniversary of the founding of the Society for the Stimulus Properties of Drugs (SSPD). The events that led to the founding of SSPD with its first official meeting on June 3, 1978, in Baltimore, MD, USA, have been described by the society's first three presidents Donald A. Overton, John A. Rosecrans, and Herbert Barry III in a special issue on drug discrimination published in *Pharmacology Biochemistry and Behavior* (Overton et al. 1999). Even prior to that first meeting of SSPD in 1978, books were beginning to appear about this new, exciting area of research that allowed behavioral pharmacologists to measure the "subjective effects" of drugs and, perhaps even more importantly, to demonstrate that the discriminative stimulus properties were related to specific pharmacological activity at the receptors of brain neurotransmitters in the central nervous system. There have been at least six different books written specially about drug discrimination and the discriminative stimulus properties of drugs (Colpaert and Balster 1988; Colpaert and Rosecrans 1978; Colpaert and Slangen 1982; Glennon and Young 2011; Ho et al. 1978; Thompson and Pickens 1971), and there also have been several special issues of journals that focused on drug discrimination studies published over the years [*Drug Development Research*, Vol. 16, 1989; *NIDA Research Monograph* 116 (DHHS pub. # ADM 92-1878), 1991; *Behavioural Pharmacology*, Vol. 2, 1991; *Pharmacology Biochemistry and Behavior*, Vol. 64 (2), 1999; *Psychopharmacology*, Vol., 2009]. Thus, there is a rich history of researchers in this field periodically coming together to present an update on the most current information about the discriminative stimulus properties of drugs. This book continues this tradition and is published as part of the *Current Topics in Behavioral Neurosciences (CTBN)* series published by Springer and is titled *The Behavioural Neuroscience of Drug Discrimination*. The goal of this volume is to provide up-to-date summaries on a number of diverse topics that encompass the current research literature for the stimulus properties of drugs.

As with any writing project like this, there are many people to thank. First and foremost, we would like to thank Bart Ellenbroek who first approached me (JHP) several years ago to see if I would be interested in editing a book on drug

discrimination. I of course said yes and then didn't think anything else about it until Bart contacted me some time later and said the project had been approved. I immediately asked Adam Prus to join me as a co-editor as he was a logical choice for a co-editor, plus I knew that this project would require a tremendous amount of time—recruiting potential authors for the various chapters and, of course, the actual editing of each chapter as they were completed. As with any writing project of this scope, there were many delays along the way and the editors and staff at Springer (K. Adeitia, Alameluh Damodharan, Susanne Dathe, Wilma McHugh, Sujitha Shiney, and Nayak SulataKumari) have displayed an amazing level of patience dealing with a large group of authors (including the co-editors!) who failed to meet deadlines much too often. Finally though, we have a finished project with 14 chapters that cover a wide diversity of topics in the field of drug discrimination. We want to thank all the contributors to each chapter as this book would not have been possible without them. Also, there are several individuals (Scott Bowen, Herbert Covington, and Richard Young) who graciously gave of their time to help out with the review process for one or more chapters. Their input was extremely valuable and helped to improve the quality and clarity of the individual chapters and of course the entire book itself. At the end of the chapter "Drug Discrimination: Historical Origins, Important Concepts, and Principles," we discuss the individual chapters and authors, and in the chapter "A Prospective Evaluation of Drug Discrimination in Pharmacology," Ellen Walker helps to put all of these diverse chapter topics into perspective.

Finally, as we noted in the first paragraph, completion of this book marks the 40th anniversary of the founding Society for the Stimulus Properties of Drugs, but it is also a bit bittersweet, as it also marks the end of this society. As often happens with small research societies, they have a natural life span—the birth of the society, the growth and development of that research field into adulthood, the maturing of that field into old age, and of course the natural ending of its existence. While we are a bit sad about this, we realize that drug discrimination has become an extremely valuable behavioral assay in the field of behavioral pharmacology and that it is utilized in many research labs around the world. It still remains the best (and perhaps the only) approach for studying the subjective effects of drugs that possess psychostimulant properties. For that reason, we are confident that drug discrimination will remain an extremely valuable research assay, with no demise in sight!

Richmond, VA, USA — Joseph H. Porter
Marquette, MI, USA — Adam J. Prus

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Contents

Drug Discrimination: Historical Origins, Important Concepts, and Principles

Joseph H. Porter, Adam J. Prus, and Donald A. Overton

Abstract Research on the stimulus properties of drugs began with studies on state dependent learning during the first half of the twentieth century. From that research, an entirely new approach evolved called drug discrimination. Animals (including humans) could discriminate the presence or absence of a drug; once learned, the drug could serve as a discriminative stimulus, signaling the availability or nonavailability of reinforcement. Early drug discrimination research involved the use of a T-maze task, which evolved in the 1970s into a two-lever operant drug discrimination task that is still used today. A number of important concepts and principles of drug discrimination are discussed. (1) The discriminative stimulus properties of drugs are believed in large part to reflect the subjective effects of drugs. While it has been impossible to directly measure subjective effects in nonhuman animals, drug discrimination studies in human subjects have generally supported the belief that discriminative stimulus properties of drugs in nonhuman animals correlate highly with subjective effects of drugs in humans. In addition to the ability of the drug discrimination procedure to measure the subjective effects of drugs, it has a number of other strengths that help make it a valuable preclinical assay. (2) Drug discrimination can be used for classification of drugs based on shared discriminative stimulus properties. (3) The phenomena of tolerance and cross-tolerance can be studied with drug discrimination. (4) Discriminative stimulus properties of drugs typically have been found to be stereospecific, if a drug is comprised of enantiomers. (5) Discriminative stimulus properties of drugs reflect specific CNS activity at neurotransmitter receptors. (6) Both human and nonhuman subjects display individual differences in their sensitivity to discriminative stimuli and drugs. (7) The drug discrimination procedure has been used extensively as a preclinical assay in drug development. This

J. H. Porter (✉)
Department of Psychology, Virginia Commonwealth University, Richmond, VA, USA
e-mail: jporter@vcu.edu

A. J. Prus
Northern Michigan University, Marquette, MI, USA

D. A. Overton
Temple University, Philadelphia, PA, USA

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chapter is the first in the volume The Behavioural Neuroscience of Drug Discrimination, which includes chapters concerning the discriminative stimulus properties of various classes of psychoactive drugs as well as sections on the applications and approaches for using this procedure.

Keywords Cross-tolerance · Discriminative stimulus · Drug development · Drug discrimination · Individual differences · State dependent learning · Stereospecific · Stimulus properties · Subjective effects · Tolerance

Contents

1 Introduction

Drug discrimination is a paradigm in which an organism learns to discriminate the pharmacological effects of a drug from the absence of drug or from the noticeably different pharmacologically effects produced by other drugs. The procedure as established today primarily relies on operant responding procedures (however, see Riley et al. 2016, this volume) and has been used in a wide variety of species, most commonly including rats, mice, and pigeons, and also in nonhuman primates and humans. Operant drug discrimination procedures require extensive training in order for an organism to accurately learn to identify the effects produced by a drug (or a combination of drugs) and the dose of that drug. The drug is referred to as a *training drug*. An appeal of this procedure is that discriminative stimulus properties of a drug can consist of those identified as *subjective*, rather than *objective*, and that the drug is a stimulus (see Catania 1971). As Catania (1971) emphasizes, discriminative control by a drug represents a *behavioral* relationship between environmental events (a drug in this case) and responses. Also, it is not necessary to understand the underlying receptor mechanisms responsible for this stimulus control, in order to understand the relationship between the interoceptive event and the response. Regardless, drug discrimination has been used extensively to study recreational and abused

substances in order to identify underlying pharmacological actions and mechanisms responsible for their subjective effects. Drug discrimination also has been utilized for studying therapeutic psychoactive drugs, such as antidepressants, anxiolytics, and antipsychotics. For a number of years, the drug discrimination paradigm has been used in both academia and industry to help elucidate the pharmacological basis of psychoactive substances. This volume, titled "*The Behavioural Neuroscience of Drug Discrimination*" as part of the *Current Topics in Behavioral Neurosciences* (CTBN) series provides reviews of the current literature for a number of either specific drugs or categories of drugs. This introductory chapter provides an overview of the historical origins of the drug discrimination procedure and discusses some important concepts and principles regarding the drug discrimination procedure.

The individual chapters in this book review the current state of the art regarding the discriminative stimulus effects of the primary classes of psychoactive drugs. The chapters highlight seminal and key findings in these areas sufficient to cover general scope of knowledge from these fields and focus on the utility of these procedures for CNS pharmacology research. Whenever possible, chapters connect the stimulus properties of drugs to mediating neuropharmacological actions (i.e., effects on specific receptor mechanisms). Moreover, the chapters in this book all document how the discriminative stimulus effects shown in animals translate to humans. Finally, by featuring leading experts in their respective areas, the chapters update and provide insight into future avenues of study with the drug discrimination paradigm.

2 Historical Origins

2.1 State Dependent Learning

As a number of excellent reviews have been written documenting the early history and concepts of the control of behavior by drugs as stimuli (e.g., Overton 1971, 1982, 1991; Schuster and Balster 1977), we will only briefly describe the historical antecedents to drug discrimination before focusing primarily on the transition from the T-maze drug discrimination developed by Overton (see Overton 1991), to the currently used operant drug discrimination procedure.

The first report of *dissociated* learning produced by drugs, later called *state dependent learning*, was by Combe (1835) who published a report of a delivery man who left a package at an incorrect address while drunk and then could not remember where he had left it until he was again intoxicated. The idea that memories might be linked to a drug state was later popularized in Wilkie Collins' classic novel "The Moonstone" (1868), which cited Combe's report as proof of the possibility. In both of these sources, the amnesic effect was apparently asymmetrical in that memories formed while drugged were unavailable without drug; however, memories formed while sober would generalize into the drug state. Later at the end of the nineteenth century, Théodule-Armand Ribot, a famous French psychologist,

developed a theory for memory retrieval in which interoceptive stimulus cues played an important role (Ribot 1882, 1891). His model predicted symmetrical amnesias with retrieval impairments after either normal to abnormal or abnormal to normal changes in body physiology. Ribot presented no new data and his theory apparently was an integral part of the intense interest in dissociation that existed in Europe throughout the last half of the nineteenth century.

The next real data about drug state dependent learning was not published until 1937 when Girden and Culler (1937) reported impaired retrieval of conditioned leg flexion responses in dogs after drug (D) to no drug (N) transitions. The effects of N to D transitions were not tested. These findings made their way into contemporary textbooks (Morgan and Stellar 1950, p 449) but seem not to have been very well integrated into the neuroscience of the time and led to only one replication attempt Gardner and McCullough (1962). It would be hard to argue that the scattered reports just described were part of a program of research about drug effects on memory retrieval. Instead, it appears that they were put in the "scientific curiosities" category and received little attention. However, the beginnings of the drug discrimination procedure can be traced back to this early work on state dependent learning conducted during the 19th and the first half of the twentieth century (Overton 1991).

2.2 Drug Discrimination

A major advancement in understanding state dependent learning came from the theories of Neal Miller, a widely respected psychologist, who argued that drug effects should act as memory retrieval cues and that laboratory experiments using a 2 × 2 design could show these effects (Grossman and Miller 1961; Miller 1957; Miller and Barry 1960). Incidentally, the 2 × 2 design employs four groups of subjects that are trained and later tested for retrieval using the drug conditions N–N, N–D, D–N, and D–D. Studies by one of the present authors (Overton 1961, 1964, 1966) also played an important role during this transition period, as it obtained convincing results and was widely read and cited. It used escape training in a T-maze drug discrimination paradigm and showed that the frank dissociative amnesias produced by high dosages were replaced by gradually acquired discriminative control at lower dosages – hence linking state dependent learning and drug discrimination phenomena.

As a better understanding of the ability of drugs to serve as stimuli (in a manner analogous to sensory stimuli) was obtained, the state dependent learning procedure evolved to produce a drug discrimination procedure. This allowed researchers to demonstrate for the first time that animals could reliably distinguish a drug state from a nondrug state and that the effects of drugs could be established as discriminative stimuli. The first drug discrimination study actually had been conducted several years earlier by Conger (1951) who used an approach/avoidance task in which rats were trained to approach when "inebriated" and to avoid when "sober," or vice versa. Thus, Conger was able to demonstrate that ethanol exerted discriminative

control over the behavior of the rats and, like other stimuli, drugs could set the occasion for responding – i.e., drugs could serve as *discriminative stimuli*. Overton (1961, 1964) further refined the drug discrimination procedure, introducing the two-choice T-maze (escape from shock), which was a symmetrical procedure in that the discriminative cue properties of the drug and nondrug conditions were demonstrated by a response selection rather than by response occurrence. Another early study reported that rats could discriminate the typical antipsychotic drug chlorpromazine from saline (Stewart 1962). Using a three-compartment test chamber (somewhat similar to a T-maze), rats were successfully trained to discriminate 4.0 mg/kg (i.p.) chlorpromazine from saline in a shock-avoidance task, and tests showed that several phenothiazines fully substituted for chlorpromazine, while the tricyclic antidepressant imipramine did not. A definitive review of this early research was published by Overton (1968). Over the next 20 years, a large number of drug discrimination studies were conducted with the T-maze procedure (see Overton 1982), but a major change in the drug discrimination procedure took place in the 1970s with the introduction of an operant task requiring rats to press response levers instead of running in a T-maze.

2.3 Two-Lever Operant Drug Discrimination

In 1968, Harris and Balster trained three rats on a two-lever multiple fixed ratio 50/differential-reinforcement-of-low rate 20 s (MULT FR 50 DRL 20 s) to discriminate DL-amphetamine from saline. After completion of discrimination testing, the rats were tested under extinction conditions in the presence of the drug cue and nondrug cue (saline). All three rats successfully acquired the amphetamine discrimination and responded primarily on the condition-appropriate lever. Harris and Balster concluded that the internal state (i.e., subjective effects of drug or no drug) of the animal controlled this responding, and that "*a more complete understanding of drug behavior interactions can be achieved by considering the* ***stimulus properties of drugs*** [emphasis added] *in addition to their traditionally emphasized pharmacological effects.*" This last statement really was both insightful and predictive as the use of the drug discrimination paradigm exploded over the next 30–40 years and became one of the most important assays for understanding the behavioral effects of drugs in vivo (see McMahon 2015). Following the Harris and Balster (1968) publication, there were a number of studies that employed this new two-lever operant drug discrimination approach, but it took several years before the procedures became more standardized. Also, as noted by Overton (1991), it soon became that two-lever operant drug discrimination procedures were more sensitive to drug stimulus effects at doses much lower than those needed in the T-maze studies and many other behavioral tests (see Kubena and Barry 1969a, b) – clearly, an advantage.

In 1971, Harris and Balster subsequently published a chapter exploring multiple two-lever drug discrimination procedures (they also tested single-lever multiple

schedules which we will not address) (Harris and Balster 1971). While they only tested a few rats under each schedule condition, they generally obtained comparable results for each drug tested in four different multiple schedules (MULT CRF CRF, MULT DRL DRL, MULT FR FR, and MULT FR EXT; CRF = continuous reinforcement, DRL = differential reinforcement of low rate 20 s, FR = fixed ratio 50, and EXT = extinction). One obvious advantage of the FR operant schedule was that it engendered higher response rates, which could be advantageous when testing was conducted under extinction conditions.

Kubena and Barry (1969a, b) subsequently demonstrated that the two-lever drug discrimination procedure could be used not only to train rats to discriminate subjective drug effects but also that novel drugs could be tested to determine if they shared discriminative stimulus properties with the training drug. Kubena and Berry (1969b) trained rats to discriminate either alcohol (1,200 mg/kg) or atropine sulfate (10 mg/kg) from saline in a two-lever drug discrimination procedure using a variable interval (VI) 1 min food reinforcement schedule. In the alcohol-trained rats, they found that pentobarbital, chlordiazepoxide, and chloral hydrate shared discriminative stimulus properties with alcohol producing almost complete alcohol-appropriate lever responding at the higher tested doses (i.e., full substitution). In the atropine-trained rats, scopolamine produced full substitution for atropine producing 100% atropine-appropriate lever responding at a dose of 1.0 mg/kg. This study demonstrated several useful properties of the two-lever operant drug discrimination. First, similar to Overton (1961) this study demonstrated that *the discriminative stimulus properties of drugs are mediated primarily by their central nervous system effects* as atropine's discriminative cue did not generalize to atropine methyl bromide, which does not cross the blood–brain barrier (i.e., it only has peripheral nervous system effects). Second, *the ED_{50} values for the dose–effect curves in the operant drug discrimination procedure were much lower than those seen in studies that used the T-maze drug discrimination procedure*. This suggested that the operant two-lever procedure was more sensitive to the behavioral effects of drugs than the T-maze procedure. Third, having response requirements for both drug- and saline-appropriate responding that are equivalent and physically adjacent is an advantage, as this makes it easier to measure the non-discriminative stimulus properties of a drug (e.g., decreasing response rates because of sedative effects).

A series of studies by Colpaert in the 1970s played a major role in helping to demonstrate the value of the two-lever operant drug discrimination procedure and to standardize the two-lever operant drug discrimination procedure. For example, Colpaert and Niemegeers (1975) trained rats to discriminate the narcotic (opioid) fentanyl (0.04 mg/kg, s.c.) from vehicle in a two-lever drug discrimination procedure using a fixed ratio (FR) food reinforcement schedule in which a food pellet was delivered after every tenth response on the condition-appropriate lever (responses on the incorrect lever had no consequence). Then, they did substitution tests with four opioids (dextromoramide, phenoperidine, piritramide, and morphine) and found that the fentanyl cue fully generalized to each of these drugs. In contrast, the neuroleptic haloperidol did not generate fentanyl-appropriate responding. Thus, this study was able to demonstrate that the "narcotic" cue produced by fentanyl generalized to other

opioids, but not to a drug from another behavioral classification (i.e., the neuroleptic haloperidol) – this showed that the discriminative stimulus properties of a drug appeared to be specific to drugs in a single pharmacological category. This study also was important as it helped to make the use of FR schedules the standard approach in two-lever operant studies. Colpaert et al. emphasized that response rates could reveal drug-induced stimulatory or inhibitory effects, while the animal's lever selection indicated difference in the drug-induced stimulus. In a subsequent study, Colpaert et al. (1975) were able to further confirm the specificity of the "narcotic" cue in 0.04 mg/kg fentanyl-trained rats and demonstrated dose-dependent generalization curves for five narcotic drugs: dextromoramide, fentanyl (the training drug), fentatienil (Sufenta® is a synthetic opioid analgesic that is more potent than its parent drug, fentanyl), morphine, and piritramide, which fully substituted for the fentanyl cue and significant reductions in responding at the highest dose tested for each drug. In contrast, the nonnarcotic drugs amphetamine, atropine, caffeine, cocaine, chlordiazepoxide, chlorpromazine, desipramine, dexetimide, haloperidol, imipramine, isopropamide, LSD, mescaline, nicotine, pentobarbital, and spiperone did not engender fentanyl-appropriate responding.

An important paper by Shannon and Holtzman in 1976 helped to lay the groundwork for establishing many of the standard approaches for studying drug effects in the two-lever operant procedure and also demonstrated the utility of the two-lever drug discrimination procedure for understanding the pharmacology underlying the discriminative stimulus properties of a drug. They trained male rats to discriminate 3.0 mg/kg morphine (s.c.) from saline in a shock-avoidance procedure (thus, the rats were not food deprived) and between 50% and 60% of the rats acquired the discrimination with greater than 90% accuracy in 6–8 weeks of training. In this study, they demonstrated a number of important features about the discriminative stimulus properties of morphine: (1) Morphine's discriminative cue was *time-dependent* with full generalization being observed within 30 min after injection and lever choice returning to the saline lever by 3.5 h after injection, (2) morphine's discriminative stimulus was *dose dependent* as they found that 0.1 mg/kg produced saline-appropriate responding while 3.0 (training dose) and 10 mg/kg produced greater than 90% morphine-lever responding (a 100-fold range), (3) morphine's discriminative cue was *pharmacologically specific* as the narcotic antagonist naloxone tested with morphine produced a rightward shift in morphine's generalization curve, (4) morphine's discriminative cue was *stereoselective* as inactive isomers of morphine and levorphanol (thebaine and dextrorphan, respectively) did not produce morphine-appropriate responding, and (5) finally, they demonstrated *cross-tolerance* to morphine's discriminative cue with methadone and the lack of cross-tolerance to pentobarbital.

Thus, by the mid-1970s the drug discrimination procedure was being used by increasing numbers of behavioral pharmacologists. As described by Overton, Rosecrans, and Barry, this increased interest in the drug discrimination paradigm led to the creation of the Society for the Stimulus Properties of Drugs (SSPD) in 1978 with the first official meeting being held that year in Baltimore, Maryland in conjunction with the CPDD (College on Problems of Drug Dependence) meeting (Overton et al.

1999). SSPD continued to hold yearly meetings until its last official meeting in 2012 in New Orleans, Louisiana. There were an increasing number of studies utilizing the drug discrimination procedure that grew exponentially through the late 1980s and peaked in 1998 with a little over 200 publications that year, followed by a subsequent decline (Bolin et al. 2016a, this volume; McMahon 2015; Stolerman et al. 1989).

There have been a few theories regarding explanation for the recent decline in the number of drug discrimination studies. One explanation is offered by McMahon (2015). Much of the drug discrimination literature has focused on drugs of abuse and many studies have used the drug discrimination assay to help determine abuse liability of new compounds. The drug self-administration paradigm appears to be replacing its use to a certain extent. As McMahon describes, self-administration certainly has greater face validity than drug discrimination with regard to drug-taking behavior in that the animals have to "work" in order to obtain the drug. Furthermore, there has been a downsizing of preclinical neuropharmacological research by many pharmaceutical companies in recent years. This has further contributed to a decline in this line of research. Despite this, drug discrimination remains a valuable tool for preclinical behavioral research.

3 Important Concepts and Principles of the Drug Discrimination Paradigm

We will not try to provide a detailed methodology for how to conduct drug discrimination studies, as there are several excellent articles/book chapters, which have been written and provided comprehensive details of training and testing methods for both human (Bolin et al. 2016b) and nonhuman (Solinas et al. 2006; Young 2009; Glennon and Young 2011a) drug discrimination studies. Rather, this section focuses on a number of important concepts and principles inherent to the drug discrimination paradigm that make it such a valuable preclinical assay for studying in vivo behavioral effects of drugs and relating those effects to specific pharmacological mechanisms.

3.1 A Method to Measure Subjective Effects

One of the most important questions to ask about drug discrimination is *what does it measure*? One commonly held assumption has been that the "*discriminative stimulus effects of drugs may be based entirely or in part upon their* ***subjective effects*** [emphasis added]" (Balster 1988). Balster further argues that understanding the underlying neural (pharmacological) mechanisms of these discriminative stimulus effects should aid in the understanding of the neural mechanisms of subjective

experiences and mood states in humans. While there are procedures for assessing and quantifying verbal reports of drugs' subjective effects in humans, verbal reports obviously cannot be obtained from nonhuman animals. This is where the drug discrimination procedure has proven to be so valuable as it allows us "*to ask*" animals "how they perceive (*feel*)" the subjective effects of drug administration. Drug discrimination is the only procedure known to the current authors to allow this unique insight into the subjective effects of drugs in animals. For example, Schuster and Johanson (1988) provided a nice review of the relationship between discriminative stimulus properties and subjective effects of drugs in both human and nonhuman studies. In experienced drug users, the subjective effects of psychotropic drugs have been assessed to help evaluate their abuse potential by comparing these effects to the subjective effects produced by known drugs of abuse. Human drug discrimination studies (e.g., Chait et al. 1985, 1986; see Bolin et al. 2016a, this volume) have helped to demonstrate that the discriminative stimulus effects of drugs correlate highly with subjective effects as assessed by verbal reports. Although Preston and Bigelow (1991) caution that "*there is a relationship* [between subjective and discriminative drug effects in human subjects]*, though not a simple one, and that the nature of the relationship is likely to be influenced by the procedural details of specific drug discrimination training and testing paradigms*." Schuster and Johanson (1988) conclude that it is very reasonable to assume that these two processes are similar in nonhuman animals. This is what makes the drug discrimination procedure so valuable for studying the subjective effects of both known and novel drugs.

Colpaert argues that since the morphine discriminative cue is due to its central narcotic action (i.e., CNS effects as opposed to peripheral effects), drug discrimination provides "*an original means by which to investigate subjectively experienced drug effects*" (Colpaert and Niemegeers 1975; see also Colpaert et al. 1975). Colpaert extended this idea to state "*that the discriminative stimulus properties of drugs, as assessed by this animal method* [i.e. drug discrimination], *may be relevant to subjectively experienced drug effects in humans*" (Colpaert et al. 1976). Other researchers in this newly emerging field of discrimination shared this viewpoint. Hirschhorn and Rosecrans (1976) stated that "*The observation that certain drugs can serve as discriminative stimuli for laboratory animals . . . demonstrates that animals can distinguish the effects of these drugs from the non-drug condition and suggests a possible method by which* ***subjective drug effects*** [emphasis added] *can be studied in animals*." Shannon and Holtzman (1976) argued that the results of their two-lever morphine discrimination study with rats "*suggest that the component of action of morphine that enables it to function as a discriminative stimulus in the rat is analogous to the component of action of morphine responsible for producing subjective effects in man*." Thus, although definitive proofs may still be elusive, there has been widespread agreement that the drug discrimination procedure provides a unique opportunity to measure the *subjective effects* of drugs by studying their discriminative stimulus properties.

3.2 Classification of Drugs

In addition to the ability of the drug discrimination procedure to measure the subjective effects of drugs, it has a number of other strengths that help make it such a valuable preclinical assay. One of these is that drug discrimination can be used to create a *classification of drugs based on shared discriminative stimulus properties*. Herbert Barry, III was one of the first to stress the utility of the drug discrimination procedure for classification of drugs according to their discriminable effects (Barry 1974). Reviewing findings from the drug discrimination literature that included both T-maze and early operant procedures, Barry summarized that the study of the discriminative stimulus properties of a large number of drugs has identified several categories including: (1) central sedatives (e.g., barbiturates and minor tranquilizers like chlordiazepoxide), (2) central anticholinergics (specifically antimuscarinic drugs), (3) nicotine, (4) marihuana (Δ^9-THC), and (5) hallucinogens (e.g., mescaline and LSD). Not surprisingly, much of the focus in the drug discrimination field has been on drugs of abuse as it was hoped that the drug discrimination paradigm would provide a unique insight into subjective effects of drugs that could relate to the abuse potential of drugs in humans. While this has been realized to a great extent, Barry stressed in his early paper the need to develop uniform procedures in the drug discrimination field. As described above (Sect. 2), the introduction of the two-lever operant drug discrimination procedure (primarily with FR schedules of reinforcement) answered this need for the most part, although as Barry (1974) pointed out there is a "*special need for the development of techniques for more rapid training of drug discrimination in rats and other laboratory animals*." This objective still has not been realized with operant drug discrimination procedures; although, a more rapid approach has been developed utilizing the "*conditioned taste aversion discrimination procedure*" (see Riley et al. 2016, this volume).

Classification of drugs with the drug discrimination procedure has been a major use of this procedure over the years. In the 1970s, as discussed above in Sect. 2, Colpaert and Niemegeers (1975) and Colpaert et al. (1975) utilized drug discrimination to identify the specificity of the stimulus properties of narcotic drugs (fentanyl was the training drug); however, the drug discrimination procedure can also be used to classify drugs for other behavioral classifications. For example, Porter et al. (2000) trained rats to discriminate a low dose of the atypical antipsychotic clozapine (1.25 mg/kg, i.p.). As shown in Table 1, all but two of the atypical antipsychotic drugs tested fully substituted for clozapine (i.e., they generated >80% clozapine-appropriate responding) and one of those produced partial substitution (>60% and <80% clozapine-appropriate responding). In contrast, none of the four typical antipsychotics fully substituted for clozapine, although thioridazine did produce partial substitution. These studies demonstrate the usefulness of the drug discrimination procedure for assigning drugs to different categories.

Table 1 Results of generalization testing in rats trained to discriminate a low dose (1.25 mg/kg) of the atypical antipsychotic clozapine form vehicle (adapted from results in Porter et al. 2000)

Test drug	Maximum percentage of clozapine-lever responding	Level of substitution[a]
Atypical antipsychotics		
Clozapine (training drug)	96.7% at 5.0 mg/kg	Full
Olanzapine	90.3% at 1.0 mg/kg	Full
Sertindole	99.8% at 5.0 mg/kg	Full
Risperidone	87.1% at 0.5 mg/kg	Full
Quetiapine	66.4% at 10.0 mg/kg	Partial
Remoxipride[b]	23.1% at 4.0 mg/kg	No
Typical antipsychotics		
Chlorpromazine	27.9% at 1.0 mg/kg	No
Fluphenazine	29.5% at 0.25 mg/kg	No
Thioridazine	74.3% at 5.0 mg/kg	Partial

[a]Level of substitution: Full = >80% drug lever responding (DRL); Partial = >60–<80% DLR; No = <60% DLR

[b]Although remoxipride is typically classified as an atypical antipsychotic, it is sometimes considered to be a typical antipsychotic (see Nadal 2001); lack of full or partial substitution for clozapine supports this conclusion

3.3 Tolerance and Cross-Tolerance

The phenomenon of *tolerance* to effects of drugs after repeated (chronic) administration has been common knowledge for a long time. As defined on the National Institute on Drug Abuse (NIDA) website (https://www.drugabuse.gov/publications/teaching-packets/neurobiology-drug-addiction/section-iii-action-heroin-morphine/6-definition-tolerance), "*When drugs such as heroin are used repeatedly over time, tolerance may develop. Tolerance occurs when the person no longer responds to the drug in the way that person initially responded. Stated another way, it takes a higher dose of the drug to achieve the same level of response achieved initially. For example, in the case of heroin or morphine, tolerance develops rapidly to the analgesic effects of the drug*." They also point out that tolerance is not the same thing as addiction, although addiction may occur to drugs that produce tolerance.

The drug discrimination procedure requires repeated administration of drugs over long periods of time (usually months of training and testing), yet the discriminative stimulus properties of drugs typically remain very stable and no evidence of tolerance or sensitivity is usually seen. For example, Colpaert et al. (1976) trained rats to discriminate 0.04 mg/kg fentanyl (i.p.) from saline and then over a period of 17 weeks fentanyl or morphine generalization curves were obtained. During each week, the rats received either two or three doses of fentanyl and/or saline (five injections each week) as part of the training regimen. The ED_{50} values for these generalization curves did not change over the 4-month period. However, the same rats used in the drug discrimination experiments did develop a marked tolerance to

the analgesic effects of fentanyl and morphine. Based on these results, the authors concluded that tolerance did not develop to the discriminative stimulus properties of narcotic analgesics.

However, under the right testing conditions, it is possible to demonstrate tolerance to drugs in the drug discrimination paradigm. Young (1991) has provided an excellent review of the conditions required to demonstrate tolerance in the drug discrimination procedure. After establishing morphine (3.2 mg/kg) as a discriminative stimulus in rats, training and testing are suspended and then the subjects were treated daily with various doses of morphine for approximately 2 weeks (varied across the studies she reviewed). Tolerance to morphine was dose dependent as low doses (3.2 or 10 mg/kg) produced little or no tolerance (i.e., the generalization curve did not change from baseline); however, the generalization curve displayed increasingly greater rightward shifts (increased tolerance) as the morphine dose was increased (maximum of 17.8 mg/kg, 2×/day). This tolerance disappeared after 3–5 days of suspending morphine treatments. Other studies she reviewed found that tolerance increased as a function of the length of morphine treatment (up to 2 weeks) and that *cross-tolerance to methadone also was evident*. (Cross-tolerance occurs when tolerance to a drug's effects produces tolerance to another drug's effects. These drugs typically belong to the same classification group and often affect the same receptor mechanisms.)

While most of the studies examining the phenomena of tolerance and cross-tolerance to the discriminative stimulus properties of drugs have focused on drugs of abuse, a series of studies also have shown tolerance and cross-tolerance with antipsychotic drugs in several drug discrimination studies. Goudie et al. (2007a) first established clozapine (5.0 mg/kg, i.p.) as a training drug in rats and determined a dose–effect curve (DEC1). Then, training and testing were suspended for 10 days and then a second DEC2 was determined. Finally, a third DEC3 was determined after a 10-day "wash-out" period during which no drug was administered and testing and training were suspended. Results revealed a significant rightward shift after the 10 days of repeated clozapine dosing (5.0 mg/kg, 2×/day) – i.e., *tolerance* to clozapine's discriminative cue was obtained. Following the 10 days of no drug treatment, the tolerance to clozapine's cue was lost and DEC3 was similar to DEC1. Using the same procedures, *cross-tolerance* was obtained with cyproheptadine (an anti-allergy/appetite stimulant), which has a binding profile very similar to clozapine. A second study by Goudie et al. (2007b) reported similar findings (cross-tolerance) with the atypical antipsychotic olanzapine and the compound JL13 (a clozapine congener). Goudie et al. concluded that the tolerance between these compounds provides a further demonstration of shared mechanisms of action. Wiebelhaus et al. (2011) used a similar procedure and demonstrated that repeated dosing with N-desmethylclozapine (major active metabolite of clozapine) and N-desmethylolanzapine (major active metabolite of olanzapine) produced cross-tolerance to clozapine (2.5 mg/kg, s.c.) in C57BL/6 mice. Cross-tolerance between these two metabolites and the atypical antipsychotic clozapine was interpreted as evidence that the discriminative stimulus properties of all three compounds shared common underlying pharmacological mechanisms.

It should be noted that Colpaert (1995) has argued that studies reporting tolerance to the discriminative stimulus of opiate drugs have in fact *not* demonstrated tolerance, although we feel that the articles discussed above (and others not cited in this review) have demonstrated tolerance. It should be noted, however, that it does appear to require specific testing conditions in order to demonstrate tolerance to the discriminative stimulus properties of drugs. While it is beyond the scope of this chapter to explore these issues thoroughly, the interested reader is encouraged to read Colpaert's (1995) review article.

3.4 Stereospecificity of Discriminative Stimulus Effects

Another important aspect of drug effects that is often not addressed in drug discrimination studies is the *stereospecificity* of the training drug. Glennon and Young (2011b) devoted an entire chapter to this topic and provided many examples of this. As they discuss, many drugs are composed of enantiomers (isomers) in a 50–50 composition, and, unless otherwise stated in a study, it should be assumed that the racemic (+) form of the drug is being used. As Glennon and Young state, "*Structural isomers are chemical entities with identical empirical formulas that differ in the nature or sequence of their atoms*." Importantly, these isomers can differ in terms of their pharmacological effects or to the extent that they are responsible for the discriminative stimulus properties of the racemic drug (see Glennon and Young 2011b for full discussion on this topic). An example of this is shown in Fig. 2. Donahue et al. (2014) trained mice to discriminate the (*S*)-isomer (10 mg/kg, s.c.) of the atypical antipsychotic drug amisulpride from vehicle. In substitution tests with *rac*-amisulpride and the (*R*)-isomer, they found that rac-amisulpride was about 3 times less potent than (*S*)-amisulpride and that (R)-amisulpride was about 10 times less potent than (*S*)-amisulpride in producing (S)-amisulpride-like responding. Figure 1 shows significant rightward shifts in the dose–response curves for *rac*-amisulpride and (*R*)-amisulpride relative to (*S*)-amisulpride. This demonstrated that the discriminative stimulus effects of amisulpride are *stereoselective* and that the (*S*)-isomer contributes more to the stimulus properties of *rac*-amisulpride than does the (*R*)-isomer (see Donahue et al. 2017 for additional confirmation of this finding). Interestingly, the potency relationships between (*S*)-, (*R*)-, and *rac*-amisulpride suggested that the stimulus effects of amisulpride could be mediated, at least in part, by activity at dopamine receptors as these potency relationships were somewhat similar to those reported in binding studies. These studies found that (*S*)-amisulpride is approximately 2 times and 20–50 times more potent than *rac*-amisulpride and (*R*)-amisulpride, respectively, with regard to binding affinity to dopamine $D_{2/3}$ receptors (Castelli et al. 2001; Marchese et al. 2002a, b). Thus, stereochemistry of a drug can be an important aspect of understanding the discriminative stimulus properties of a drug. The isomers of a drug may both contribute to the discriminative stimulus properties of the drug with one isomer being more potent than the other (i.e., stereoselectivity), or one of the isomers may have similar properties to the parent drug and contribute to its discriminative stimulus, while

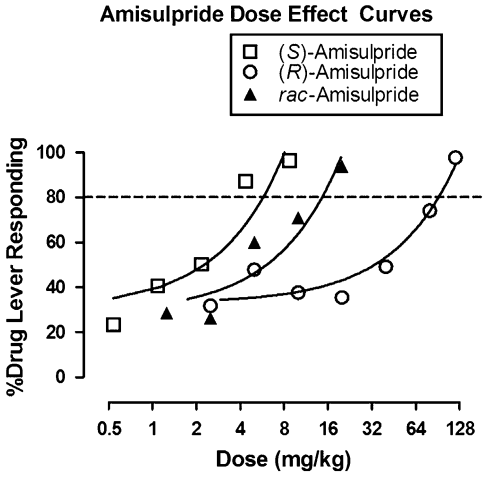


Fig. 1 This figure presents a direct comparison of the dose–response curves of %drug lever responding (DLR) for rac-amisulpride and its (S)- and (R)-enantiomers with regression lines. Doses for (S)-amisulpride were adjusted to the base form for direct comparison (ED50¼ 1.57 mg/kg [95% C.I.¼1.14–2.15 mg/kg]). From Donahue et al. (2014) – reproduced with permission

the other does not (i.e., stereospecificity) (for more complete discussion of this, see Chapter 4 in Glennon and Young 2011b).

3.5 *Receptor Mechanisms and Discriminative Stimulus Effects*

In 1988, an entire book was devoted to *transduction mechanisms of drug stimuli* (Colpaert and Balster 1988). A major theme of this book was that discriminative stimulus properties of drugs reflect specific CNS effects at *neurotransmitter receptors*. As Balster (1988) states "*If discriminative effects are related to subjective effects, then it seems reasonable to hope that studies of the neural mechanisms for these effects may lead us toward an understanding of the neural mechanisms of some of the subjective experiences and mood states that are the basis of our perception of drug effects*." Balster concludes with "*Studies of discriminative stimulus properties of drugs and their mechanisms of transduction can provide us important insights into basic brain-behavior relationships*." An early example of this was a study by Rosecrans and Glennon (1979) in which they used drug cues to study psychoactive mechanisms by comparison to other drugs (i.e., substitution tests) and by determining if the drug cue could be antagonized (i.e., blocked) with specific receptor antagonists. For example, in morphine-trained rats they demonstrated that methadone was equally potent in producing a similar dose-dependent generalization curve;

whereas, meperidine was significantly less potent as indicated by a rightward shift in the generalization curve. In antagonism studies, they found that the discriminative stimulus of LSD could be antagonized in a dose-dependent manner (i.e., greater antagonism with increasing doses) by the serotonin antagonist (BC105). Perhaps more interestingly, they presented findings comparing serotonin binding affinities (data presented as pA2 values from rat fundus assays) in a series of tryptamine analogs to the ability of these compounds to substitute for 5-OMeDMT (1.5 mg/kg training dose). As shown in Fig. 2 from that study, there was a strong correlation between the pA2 values and the equivalent dose at which each compound substituted for 5-OMeDMT, which is a hallucinogen with strong affinity for serotonin 5-HT2 and 5-HT1A receptors. These data clearly demonstrated that the discriminative stimulus properties of 5-OMeDMT were related to its binding affinity to serotonin receptors. In a study in which two separate groups of Sprague-Dawley rats were trained to discriminate either the atypical antipsychotic clozapine (5.0 mg/kg, i.p.) or the muscarinic antagonist scopolamine (0.5 mg/kg, i.p.) from vehicle, it was found that complete *cross-generalization* occurred between clozapine and scopolamine, indicating a shared underlying mechanism for their respective discriminative stimuli. In addition, only drugs that display high binding affinities for muscarinic cholinergic

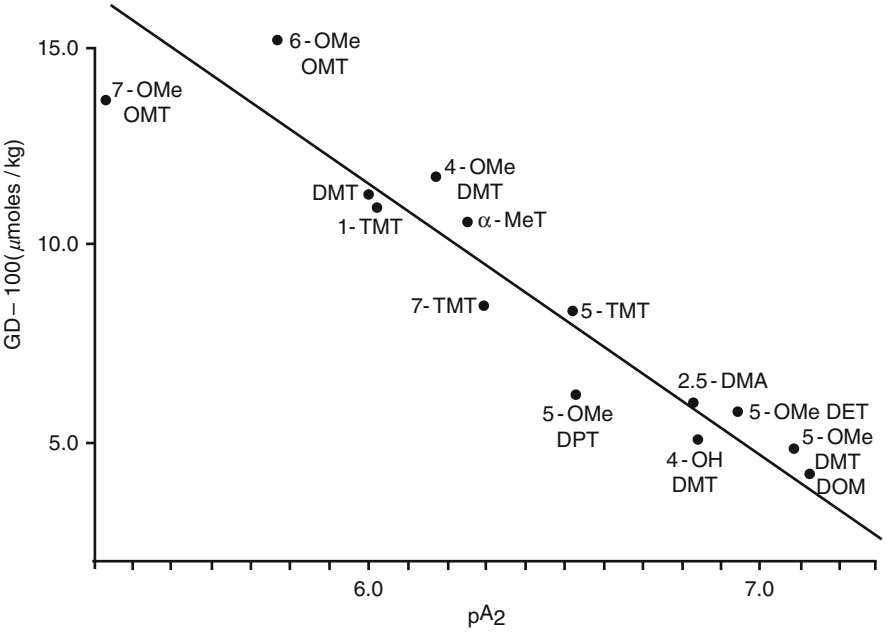


Fig. 2 This figure presents the correlation between the discriminative stimulus (DS) properties and pA2 values of a series of tryptamine and phenylisopropylamine analogs. The GD-100 value represents the equivalent dose at which each compound generalized with the training dose of 5-OMeDMT (1.5 mg/kg) when it was used as the DS. Each drug was administered at various doses 15 min prior to being placed in the operant chamber for a 15-min test session. From Rosecrans and Glennon (1979) – reproduced with permission

receptors substituted for these two training drugs. Based on these results, the authors concluded that antagonism of muscarinic receptors (especially M_1) plays an important role in the discriminative stimulus properties of clozapine in rats (Kelley and Porter 1997). In contrast, in C57BL/6 mice, clozapine's discriminative cue appears to be mediated by antagonism of serotonergic 5-HT_2 receptors and α_1 adrenoreceptors (Philibin et al. 2005, 2009). Thus, it is often possible to ascertain the underlying receptor mechanisms that mediate the discriminative stimulus properties of a drug. However, as these studies show, these mechanisms may differ across species. Therefore, some caution must be exercised when making inferences across different species, including humans.

3.6 *Individual Differences Between Subjects*

An important, but understudied topic in drug discrimination research concerns *individual differences* between subjects in their sensitivity to the discriminative stimulus properties of drugs. These differences are often reflected in the number of training sessions required for individual subjects to meet the discrimination criterion – i.e., some subjects will acquire the discrimination in fewer training sessions than other subjects. The first operant study to systematically explore the importance of the speed of acquisition of the discrimination was by Martin Schechter in 1983. Twelve male Sprague-Dawley rats were trained to discriminate 0.16 mg/kg apomorphine from saline with responding reinforced according to an FR 10 reinforcement schedule. Half of the rats acquired the discrimination in a mean of 22.5 sessions (*early learners*) and the other half in a mean of 44.2 sessions (*late learners*) (significantly different, $p < 0.001$). When apomorphine generalization curves were established, the early learners had an $ED_{50} = 0.01$ mg/kg; whereas, the late learners had an $ED_{50} = 0.07$ mg/kg, which represented a 3.9-fold rightward shift in the generalization curve (the dose–response curves were parallel). Thus, the early learning group was more sensitive to apomorphine's discriminative stimulus than was the late learning group. While it has been well established that higher training doses result in higher ED_{50} values for the dose–response curves (see review by Stolerman et al. 2011), Schechter's (1983) study was the first to demonstrate that sensitivity to the training drug's discriminative cue also could affect the ED_{50}.

A second study by O'Neal et al. (1988) examined how the rate of acquisition of Δ^9-THC (delta-9-tetrahydrocannabinol) discrimination reflected sensitivity to Δ^9-THC's discriminative stimulus. Male Sprague-Dawley rats were trained to discriminate 3.0 mg/kg Δ^9-THC from saline in a two-lever operant discrimination task (FR 10) and after acquisition of the Δ^9-THC discriminative cue, the rats were divided into two groups using a median split – slow learners and fast learners. For the slow learners, the mean number of sessions to criterion (STC) = 50.0; for the fast learners, the STC = 27.3 (significantly different, $p < 0.001$). Similar to results found in the Schechter's (1983) study, the slow learners displayed a rightward shift in the Δ^9-THC generalization curve with an $ED_{50} = 1.63$ mg/kg; whereas, the ED_{50} for the fast

learners = 0.77 mg/kg. Thus, the fast learners displayed a greater sensitivity to Δ^9-THC, replicating the greater sensitivity to apomorphine shown by the fast learners in the Schechter's (1983) study. We have not been able to find additional studies that have examined the relationship between speed of acquisition and the subsequent sensitivity of individual subjects to a drug's discriminative cue. Nonetheless, both of these studies suggest that reporting the number of sessions required to reach the training criteria should be information routinely provided in publications.

Bevins et al. (1997) reported that individual differences in rats in the sensitivity to amphetamine in several behavioral assays (novelty-induced activity, novelty-induced place preference, novel-object interaction, and amphetamine-induced activity) were related to differences in amphetamine discrimination. For example, rats more sensitive to the activating effects of amphetamine were also more sensitive to amphetamine in the drug discrimination assay. Individual differences in *human subjects* also have been shown in nicotine drug discrimination studies. Perkins (2011) summarizes some of the factors that contribute to individual differences in human nicotine drug discrimination studies. For example, these studies find that women, generally, are less sensitive to nicotine's discriminative stimulus properties as reflected in more difficulty in acquiring the cue or showed flattened generalization curves. Individual differences in animal studies with nicotine drug discrimination also have been shown that may be related to genetic differences (e.g., Quarta et al. 2009). Finally, Morgan and Picker (1996) reported three- to tenfold differences in the lowest doses of several opiates that would substitute for morphine in rats trained to discriminate morphine (3.0 mg/kg) from vehicle in a two-lever drug discrimination study. Individual differences were also observed in the antinociceptive effects of these opiates in a hot water tail-withdrawal procedure. These authors concluded that these individual differences between subjects are probably determined in large part by the relative efficacy of these drugs at the *mu* opioid receptor.

Finally, it is also possible that differential sensitivity among subjects to the discriminative stimulus properties of drugs may reflect the fact that different subjects may "tune" into different components of a cue. It has been well established that "compound" discriminative stimuli can be demonstrated with drug mixtures as the cue (see review by Stolerman et al. 1999). However, it is also possible for a single drug to have a *compound* discriminative cue. For example, in rats trained to discriminate ethanol from water, asymmetrical generalization of ethanol to gamma-Aminobutyric acid (GABA) enhancers (e.g., chlordiazepoxide), to N-methyl-D-aspartate (NMDA) antagonists (e.g., dizocilpine [MK-801]), and to serotonin (5-HT) agonists (e.g., trifluoromethylphenylpiperazine) was found. Stolerman et al. (1999) concluded from these studies that their finding supported the concepts of ethanol having a compound stimulus (see also Grant 1999), since ethanol generalized to drugs of more than one pharmacological classification. It certainly seems reasonable to assume that subjects might attend to one or more components of a drug's pharmacological actions, which make up its compound cue and might explain individual differences in acquisition to a drug's discriminative cue.

3.7 Drug Development

As a preclinical behavioral assay, drug discrimination has proven to be a useful tool. For example, clozapine is an atypical antipsychotic drug that is considered to be the "gold standard," prototypical of the second generation of antipsychotic drugs and it remains the standard by which other atypical antipsychotic drugs are compared (Hippius 1991; Porter and Prus 2009). When the antipsychotic olanzapine was being developed by Eli Lilly and Company, Moore et al. (1992) published an article on the behavioral pharmacology of olanzapine. One of the behavioral assays employed in that study was two-lever drug discrimination in which clozapine 5.0 mg/kg, i.p. was trained as a discriminative stimulus. Olanzapine fully substituted for clozapine's cue, indicating that olanzapine's discriminative stimulus properties were similar to those of clozapine. Based on these results, and results from a number of other behavioral assays used in this study, the authors concluded that olanzapine would have the profile of an atypical antipsychotic drug (like clozapine). Olanzapine was later approved by the FDA in 1996 for treatment of schizophrenia.

Drug discrimination has been frequently used by pharmaceutical companies (e.g., Millan et al. 1999) and in academia (e.g., Burgdorf et al. 2013) to help characterize the behavioral pharmacology of novel compounds and by government agencies like the Drug Enforcement Agency (DEA) to aid in scheduling the abuse liability of drugs (see Ator and Griffiths 2003; Balster and Bigelow 2003). In addition to the atypical antipsychotic drug olanzapine (see above), another good example is the atypical antipsychotic risperidone. Colpaert (2003) has written an excellent review of the discovery process for risperidone and how important the study of subjective effects in laboratory animals was to this process. He concluded that "*the pathway to risperidone chiefly cut across the field of in vivo pharmacology, and in particular behavioral pharmacology, underscoring the unique contribution of the field to drug discovery*." In 2002, the In Vivo Pharmacology Training Group published a commentary on "*The rise and fall of in vivo pharmacology*." In this article, they stated "*Pharmacology is, by definition, the study of the mechanism of action of drugs, and requires a knowledge and understanding of responses to drugs induced both in vitro and in vivo. Such analysis of drug action is needed to transform molecular or cellular discoveries into clinical practice and, equally, to identify the molecular questions that arise from clinical observations. These studies are essential because responses observed in vitro can be magnified, diminished or totally different in the more complex integrated system. . . .. . .This article . . .. . . outlines why in vivo work is vital for the analysis of drug action and for the discovery and development of new therapeutic agents*." (In Vivo Pharmacology Training Group 2002). We concur with these conclusions and recognize the utility and value of preclinical behavioral assays in the drug development process. Behavioral (in vivo) assays (like drug discrimination) are just as important as in vitro assays for this process and the two approaches go hand-in-hand in the discovery and development of new therapeutic drugs.

4 Summary and Overview of This Volume

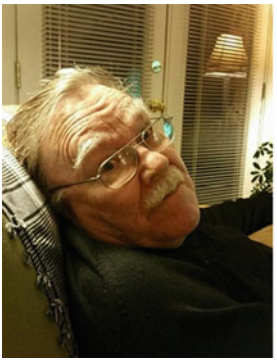
The current chapter (Chapter 1 in Part 1, this volume) provided a brief overview of the historical origins of the drug discrimination procedure and described how its beginnings can be traced to state dependent learning, and then how it transitioned from the first drug discrimination studies in a T-maze task in the 1960s to a two-lever operant procedure in the 1970s. Then, we discussed how the discriminative stimulus properties of drugs are believed in large part to reflect the subjective effects of drugs and that drug discrimination studies in human subjects have generally supported the belief that discriminative stimulus properties of drugs in nonhuman animals correlate highly with subjective effects of drugs in humans. Finally, we discussed a number of other concepts and principles that help make drug discrimination a valuable preclinical assay.

The chapters in Part 2 of this volume review the current state of the art regarding the discriminative stimulus effects of the primary classes of psychoactive drugs. In Chapter 2, William Fantegrossi provides an overview of early drug discrimination work on psychostimulant drugs but also includes coverage of recent findings on the discriminative stimulus effects of bath salts. Chapter 3 provides a thorough summary about the discriminative stimulus effects of nicotine and recognizes the influential work of John Rosecrans, who is posthumous co-author of this chapter with Richard Young. The discriminative stimulus effects of ethanol are addressed extensively by Kathleen Grant's group in Chapter 4, and the chapter pays particular attention to the stimulus effects of ethanol across different species, including humans. Of particular relevance for interpreting the subjective effects of ethanol from these studies, this chapter points out the qualitatively different stimulus properties of low versus higher training doses of ethanol. In Chapter 5, Keith Shelton takes us through studies designed to evaluate the subjective effects of inhalants and devotes some emphasis to the unique methodological challenges involved in this work. In Chapter 6, Tsutomu Suzuki, with Tomohisa Mori, reviews an extensive literature on the discriminative stimulus effects of hallucinogens and dissociative anesthetic drugs, e.g., ketamine, and gives a glimpse of future directions in drug discrimination research as he associates intracellular signaling processes to the mediation of certain stimulus effects. In Chapter 7, two of the leading experts on the behavioral pharmacology of cannabinoids, Jenny Wiley and Aron Lichtman, contribute to a review on the discriminative stimulus effects of cannabinoids, which includes stimulus effects of endocannabinoids as well as synthetic cannabinoid compounds. Eduardo Butelman and Mary Jeanne Kreek, in Chapter 8, gave an up-to-date account on drug discrimination for opioid compounds and provided novel thoughts on future directions in this area. Chapter 9 is the first of two chapters that focused on the discriminative stimulus effects of drugs for mental illness. Chapter 9, written by the co-editors of this volume, along with the assistance of Kevin Webster, reviews studies evaluating the stimulus effects of antipsychotic drugs, with an emphasis on

the utility of this procedure for identifying effective antipsychotic drugs for schizophrenia. The chapter also connects reported subjective effects of antipsychotic drugs in human patients to certain receptors known to mediate stimulus effects of antipsychotics in animals. Chapter 10, also co-authored by the editors of this volume, uses the same approach to evaluate the discriminative stimulus effects of antidepressants and anxiolytics.

Part 3 of this volume, called "Approaches to Drug Discrimination," provides a variety of perspectives on ways to understand the drug discrimination procedures along with some of its applications. In Chapter 11, Steve Negus and Matthew Banks discuss analyzing drug discrimination data using pharmacokinetic–pharmacodynamic analyses. In Chapter 12, Craig Rush reviews drug discrimination studies conducted in humans and some of the methodological advantages and challenges. In Chapter 13, Anthony Riley and others from his group demonstrate how the stimulus properties of drugs can be studied using conditioned taste aversion procedures. In this volume's final chapter (Chapter 14), Ellen Walker provides commentary on the chapters in this volume and discusses new directions for the use of drug discrimination in pharmacology research. Overall, this volume on the drug discrimination provides an insightful evaluation of a wide array of critical topics in this field written by leading experts on this procedure. The editors of this volume are grateful to all of the authors who have made this a notable addition to the literature in behavioral neuroscience.

Finally, we would like to dedicate this volume to the memory of two pioneer researchers in the field of drug discrimination. Dr. John A. Rosecrans and Dr. Torbjörn U.C. Järbe were two of the early scientists in drug discrimination research who did so much to help shape this newly emerging area of research back in the 1970s and whose influence continued into this century. Their legacy and influence in this field lives on and will be remembered always. We will miss both of them.



John A. Rosecrans
(1935–2015)

Torbjörn U.C. Järbe
(1946–2017)

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Discriminative Stimulus Effects of Psychostimulants

Michael D. Berquist and William E. Fantegrossi

Abstract Numerous drugs elicit locomotor stimulant effects at appropriate doses; however, we typically reserve the term psychostimulant to refer to drugs with affinity for monoamine reuptake transporters. This chapter comprises select experiments that have characterized the discriminative stimulus effects of psychostimulants using drug discrimination procedures. The substitution profiles of psychostimulants in laboratory rodents are generally consistent with those observed in human and nonhuman primate drug discrimination experiments. Notably, two major classes of psychostimulants can be distinguished as those that function as passive monoamine reuptake inhibitors (such as cocaine) and those that function as substrates for monoamine transporters and stimulate monoamine release (such as the amphetamines). Nevertheless, the discriminative stimulus effects of both classes of psychostimulant are quite similar, and drugs from different classes will substitute for one another. Most importantly, for both the cocaine-like and amphetamine-like psychostimulants, dopaminergic mechanisms most saliently determine discriminative stimulus effects, but these effects can be modulated by alterations in noradrenergic and serotonergic neurotransmission as well. Thusly, the drug discrimination assay is useful for characterizing the interoceptive effects of psychostimulants and determining the mechanisms that contribute to their subjective effects in humans.

Keywords Discriminative stimulus • Drug discrimination • Monoamine transporter blocker • Monoamine transporter releaser/substrate • Preclinical model • Psychostimulants • Stimulus properties

M.D. Berquist and W.E. Fantegrossi (✉)
Department of Pharmacology and Toxicology, College of Medicine, University of Arkansas for Medical Sciences, 4301 West Markham Street, Slot 638, Little Rock, AR 72205, USA
e-mail: WEFantegrossi@uams.edu

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Contents

1 Introduction

This chapter reviews select experiments that have characterized the discriminative stimulus effects of psychostimulants using drug discrimination procedures. We limit our discussion to psychostimulants that primarily serve as reuptake inhibitors or substrates for release at monoamine transporters with a special emphasis on drugs that directly modulate dopamine (DA) neurotransmission (e.g., through pharmacological effects at the dopamine transporter [DAT]). Drugs that directly modulate serotonergic activity at the serotonin transporter (SERT), noradrenergic activity at the norepinephrine transporter (NET), intracellular mechanisms (e.g., intraterminal vesicular releasers), and neuronal activity via stimulation at postsynaptic dopaminergic cell-surface receptors will also be discussed. In addition, given the current interest in an emerging class of psychostimulants, the synthetic cathinones (casually referred to as "bath salts"), we will briefly discuss their discriminative stimulus effects where appropriate. Drugs that produce acute psychostimulant-like effects through other, non-monoaminergic pharmacological mechanisms (e.g., phencyclidine, caffeine, and nicotine) will not be included and we refer readers to chapters found elsewhere in this book or in other available resources.

2 Discriminative Stimulus Effects of Psychostimulant Drugs

As discussed within other chapters of this book, drug discrimination procedures are valuable for characterizing the discriminative stimulus effects of psychoactive substances. Although most psychostimulant drug discrimination studies are conducted in rodents (e.g., [1]), the substitution profiles of psychostimulants are generally consistent with those observed in human and nonhuman primate drug discrimination experiments. As such, where available, the similarities in drug substitution profiles observed across human and nonhuman drug discrimination findings will be reported in the present chapter to highlight the predictive utility of the drug discrimination assay in nonhuman subjects. The sections that follow are categorized by pharmacological effects at specific protein targets in the central nervous system. Each of the following sections will include a representative drug from the associated pharmacological class to demonstrate the utility and translatability of drug discrimination procedures. Furthermore, an overview of the experimental parameters in drug discrimination preparations (e.g., difference between partial substitution and full substitution) will not be discussed here, but drug discrimination terms and concepts will be mentioned throughout this chapter. Readers may refer to [2] within this book to glean basic concepts of drug discrimination methodology, if needed.

3 A Note on the Sex of Experimental Subjects

Most preclinical research has used males as experimental subjects and considerably fewer drug discrimination experiments have included females. Despite historical precedents for preferentially concentrating on male subjects instead of females (e.g., convenience, literature precedence), the consideration of sex as a biological variable is currently being promoted at all levels of NIH-funded research. Indeed, the NIH has recently added a policy requiring some discussion of experimental designs to study male and female animals in preclinical studies, unless sufficient justification can be given that such sex-specific inclusion would be unwarranted. In light of this new policy, we include below several drug discrimination experiments that used psychostimulants as training or test compounds and made direct comparisons of males to females in discriminative performance, but note that this area is considerably understudied in comparison to other common in vivo assays of psychostimulant effects which show sex differences, including locomotor activity [3, 4] and intravenous self-administration [5, 6].

4 Monoamine Transporter Blocker: Cocaine

4.1 Dopamine

Cocaine is a nonselective, passive reuptake inhibitor at DAT, NET, and SERT [7–9], and possesses pharmacologically-relevant binding affinities at serotonin $(5\text{-HT})_3$ receptors [10], muscarinic M_1 and M_2 receptors [11], and σ-receptors [12]. In an early drug discrimination experiment that included cocaine as the training drug, Colpaert et al. [13] reported that 1.25 mg/kg racemic amphetamine (a relatively selective DA releaser, see below) and 0.31 mg/kg apomorphine (a direct agonist at DA receptors) produced partial substitution (intermediate percent drug-lever responses) in rats trained to discriminate 10 mg/kg cocaine (a common training dose of cocaine) from saline; however, the antipsychotics haloperidol (0.08–0.16 mg/kg) and pimozide (1.25–2.5 mg/kg), which are potent dopamine antagonists, failed to block cocaine's cue. In a similar study, McKenna and Ho [14] observed that *d*-amphetamine (0.25–0.5 mg/kg) (an isomer of amphetamine with greater in vivo potency, now referred to as *S*(+)-amphetamine to denote absolute configuration) and apomorphine (0.25–0.5 mg/kg) produced complete generalization in rats trained to discriminate 10 mg/kg cocaine from saline, whereas pretreatment with 0.5 mg/kg haloperidol attenuated cocaine's cue as evidenced by a downward shift in cocaine's dose–response curve. A downward shift of a training drug's dose–response curve in the presence of an antagonist generally indicates noncompetitive (or insurmountable) antagonism (assuming the dose of antagonist is held constant and the dose of the training drug varies), although it may also indicate a sedative/motoric effect or stimulus masking (i.e., the antagonist produces an interoceptive cue that reduces/competes with the saliency of the training drug cue). Unfortunately, McKenna and Ho [14] did not present response rate data to supplement the substitution test results. Disparate findings in drug discrimination research (e.g., haloperidol weakened cocaine's discriminative stimulus effects in one study [14], but not in another [13]) are common if one considers the context under which discrimination training and testing occur; indeed, a drug's discriminative stimulus effects are not an immutable property of the drug, but are rather the result of a complex interaction of variables related to the drug's pharmacological effects, the experimental environment, and the experimental subject's learning history (e.g., contingency of reinforcement).

As mentioned previously, relatively few drug discrimination experiments have included females as experimental subjects. Nevertheless, previous research has made direct comparisons between males and females in discrimination performance of cocaine and two of these studies will now be discussed. Craft and Stratmann [15] trained male and female Sprague–Dawley rats to discriminate 5.6 mg/kg cocaine (IP) from saline using a two-lever food-maintained drug discrimination procedure. There were no significant differences in acquisition of drug stimulus control, estimates of median effective dose (ED_{50}) values of cocaine (1.0–10 mg/kg) or *d*-amphetamine (0.1–0.56 mg/kg) following substitution tests,

or in the acquisition and substitution profile when the training dose increased to 10 mg/kg cocaine. Moreover, these authors demonstrated that cocaine more potently stimulated locomotor activity in females than in males, but discrimination performance was virtually identical between sexes, indicating that a drug's locomotor effects must be considered separately from its discriminative stimulus effects.

In a later study, Anderson and van Haaren [16] trained male and female Wistar rats to discriminate 10 mg/kg from cocaine. Similar to the findings reported by Craft and Stratmann [15], there were no significant differences between males and females in acquisition of drug stimulus control (i.e., learning to discriminate the training drug from vehicle), substitution tests with cocaine (1.0–10 mg/kg), and the blocking effects of the D_1 receptor antagonist SCH-23390 (0.01–0.10 mg/kg) or the D_2 receptor antagonist raclopride (0.1–1.6 mg/kg) when each antagonist was injected prior to 10 mg/kg cocaine [16]. Based on these findings, it appears that males and females display comparable performance in discriminating cocaine's cue, and cocaine's interoceptive effects are mediated by common dopaminergic mechanisms.

The foregoing drug discrimination experiments and others (e.g., [17, 18]) have demonstrated that DA has an important role in cocaine's discriminative stimulus effects. Later research has confirmed these preliminary reports and provided further evidence that increased DA neurotransmission through DAT blockade is considered primarily responsible for mediating cocaine-like stimulus effects. For example, the selective DAT inhibitor GBR 12909 is 17-fold more potent at inhibiting DAT than SERT as measured in vitro [8]. GBR 12909 (2–16 mg/kg) produces complete generalization to cocaine in rats trained to discriminate 10 mg/kg cocaine from saline, and pretreatment with 2 mg/kg GBR 12909 potentiates cocaine's discriminative stimulus effects, as evidenced by a leftward shift in the cocaine dose–response curve [19]. In rats trained to discriminate 2.5 mg/kg cocaine from 10 mg/kg cocaine, Kleven and Koek [20] reported that pretreatment with the structurally related DAT-selective GBR 12935 potentiated the discriminative stimulus effects of 2.5 mg/kg cocaine; that is, as the test dose of GBR 12935 increased, rats shifted responding from the "low dose" 2.5 mg/kg cocaine lever to the "high dose" 10 mg/kg cocaine lever. In addition, GBR 12935 failed to fully substitute for 10 mg/kg cocaine in >50% of rats when injected prior to saline administration. It is noteworthy that GBR 12935 is approximately 78-fold more potent at inhibiting DAT than SERT as measured in vitro [8]. It is possible that the failure to fully substitute for cocaine is due to GBR 12935 possessing binding affinities to other protein receptors or membrane protein transporters [8], or perhaps low in vivo potency compared to its in vitro binding profile (see [20]). Nevertheless, rats can be successfully trained to discriminate GBR 12909 from cocaine [21], indicating that these drugs produce similar, but not identical, discriminative stimulus effects. As a final point, it is noteworthy that rats can be trained to discriminate 2.5 mg/kg from 10 mg/kg cocaine (a "dose–dose discrimination") ([20]; also see [22]). Indeed, the selected training dose of a drug is a primary determinant in its subsequent discriminative stimulus effects profile, which is unsurprising given that the magnitude of a

drug's behavioral effects occur in a dose-dependent fashion. Indeed, there are oftentimes qualitative differences (e.g., dissimilar generalization gradients) between different doses of the same drug. In any event, Kleven and Koek [20] utilized this dose–dose discrimination preparation to detect cocaine-like effects that may not occur at the common 10 mg/kg cocaine training dose. Regardless of the drug discrimination methodology used, various DA uptake inhibitors (e.g., methylphenidate, WIN 35428, indatraline) and DA releasers (e.g., cathinone, fencamfamine, and methamphetamine) all produced full substitution in rats trained to discriminate 10 mg/kg cocaine from saline [23], further indicating that altered DA neurotransmission is generally considered to be the primary mediator of cocaine's discriminative stimulus effects. In a human drug discrimination experiment, Rush and Baker [24] observed that oral methylphenidate (a fairly selective DAT inhibitor, with at least eightfold selectivity for DAT over NET, and negligible SERT affinity) produced full substitution in humans trained to discriminate an oral dose of 200 mg cocaine, while the benzodiazepine triazolam produced an expected very low cocaine-appropriate responding. At this point it is worth noting that findings from positron emission tomography (PET) experiments in humans support the foregoing role of increased dopaminergic tone due to DAT blockade in mediating cocaine's discriminative stimulus effects as measured in rodents. For example, Volkow et al. [25] observed that the self-reported high following cocaine administration (IV) was significantly correlated to DAT occupancy. Indeed, blockade of $\geq$47% of DAT was required to elicit cocaine's subjective effects in the volunteers [25].

It is therefore reasonable to speculate that increased dopaminergic tone (e.g., via inhibition of reuptake through the DAT) would necessarily lead to increases in postsynaptic receptor stimulation in the absence of factors that would limit dopaminergic neurotransmission (e.g., overexpression of catalytic enzymes, such as monoamine oxidase). In congruence with this notion, in addition to changes in overall dopaminergic tone, previous studies have demonstrated that stimulation of postsynaptic DA receptors are involved in mediating cocaine's discriminative stimulus effects. For example, Callahan et al. [26] observed that the D_2-like receptor agonist quinpirole (0.0313–0.125 mg/kg) produced full substitution for cocaine in rats trained to discriminate 10 mg/kg cocaine from saline, but the D_1-like receptor agonist SKF 38393 (5–20 mg/kg) produced only partial substitution before eliciting behavioral disruption in these subjects. In that same study, the D_2-like receptor antagonist haloperidol and the D_1-like receptor antagonist SCH 23390 (0.0063–0.25 mg/kg) reduced cocaine-lever responding when administered prior to the cocaine training dose. This study, and others not discussed here (e.g., [27, 28]), specify receptors that are involved in mediating cocaine's discriminative stimulus effects downstream from its direct pharmacological effects at DAT. For a review of DA's involvement in mediating cocaine's discriminative stimulus effects, see Callahan et al. [29].

As mentioned previously, synthetic cathinones are an emerging class of psychostimulants that increased in popularity in the early 2000s (for review, see [30]). Most of these compounds produce increases in extracellular monoamines

through intraterminal release through a monoamine transporter, or via reuptake inhibition at monoamine transporters (for review, see [31]). In a recent study, Gatch et al. [32] trained groups of rats to discriminate 10 mg/kg cocaine or 1 mg/kg methamphetamine from saline. The synthetic cathinones 3,4-methylenedioxypyrovalerone (MDPV) (0.05–2.5 mg/kg), 4-methylmethcathinone (mephedrone) (0.5–5 mg/kg), methylone (0.5–5 mg/kg), napyhrone (0.5–5 mg/kg), flephedrone (0.5–10 mg/kg), and butylone (0.5–10 mg/kg) were tested for stimulus substitution. The results revealed that all synthetic cathinones produced full substitution in both the 10 mg/kg cocaine and 1 mg/kg methamphetamine training groups, indicating that these compounds produce interoceptive effects that are comparable to prototypical psychostimulants. Similarly, mice trained to discriminate 10 mg/kg cocaine from saline fully generalized responding to racemic MDPV and its enantiomers, with S(+)-MDPV being more potent than the racemate and R(−)-MDPV being dramatically (~30-fold) less potent than the racemate [33]. Importantly, mephedrone and naphyrone also fully substituted for cocaine in those same mice [34].

It should be noted that MDPV is a potent reuptake inhibitor at DAT and NET (and is approximately sixfold more selective for DAT over NET), with negligible affinity for SERT [35], whereas cocaine is a nonselective reuptake inhibitor at DAT, NET, and SERT (e.g., [35]). As such, substitution tests with drugs that increase extracellular DA and NE content, or stimulate postsynaptic dopamine and noradrenergic receptors may produce interoceptive effects that are similar to MDPV. In a recent report by Fantegrossi et al. [36] male NIH Swiss mice were trained to discriminate 0.3 mg/kg MDPV from saline. Substitution tests were performed with MDPV (0.01–0.3 mg/kg), MDMA (0.01–0.3 mg/kg), methamphetamine (0.01–0.3 mg/kg), morphine (1–30 mg/kg), and the synthetic cannabinoid JWH-018 (0.1–3 mg/kg). MDPV, MDMA, and methamphetamine engendered >75% MDPV-appropriate responding, whereas morphine and JWH-018 produced <50% MDPV-appropriate responding. These results indicate that MDPV, a drug with pharmacological actions that are similar to cocaine, produces interoceptive effects similar to prototypical drugs of abuse.

4.2 Norepinephrine

In addition to DAT, cocaine is also a nonselective reuptake inhibitor at NET and SERT. Comparatively fewer studies have investigated the role of NE neurotransmission in mediating cocaine's discriminative stimulus effects. In an early report, Colpaert et al. [13] observed that two compounds with effects at adrenoceptors, dibenamine (an α-adrenoceptor antagonist) and propranolol (a β-adrenoceptor antagonist), failed to block cocaine's discriminative effects in rats trained to discriminate 10 mg/kg cocaine from saline, indicating that the NE-releasing effects of cocaine may not contribute to its discriminative stimulus effects, or at least that antagonism of the α- and β-adrenoceptors do not *attenuate* cocaine's discriminative stimulus effects. As mentioned above, the initial training dose and subsequent

methods used to test for substitution in drug discrimination experiments critically determine a drug's discriminative stimulus effects. Indeed, Young and Glennon [37] observed cross-substitution of cocaine and propranolol in rats that were trained to discriminate doses of either drug. That is, the β-adrenoceptor antagonist *substituted* for rather than *attenuated* cocaine's discriminative stimulus effects. In an earlier report that differentiated the role of adrenoceptors in mediating cocaine's discriminative stimulus effects, Kleven and Koek [22] demonstrated that pretreatment with the β_1/β_2-adrenoceptor antagonists (−)-propranolol and tertatolol, as well as the β_2-adrenoceptor antagonist ICI 118,551, produced high-dose lever selection (i.e., 10 mg/kg cocaine-lever selection) when administered prior to 2.5 mg/kg cocaine injection in rats trained to discriminate 2.5 mg/kg cocaine from 10 mg/kg cocaine. That is, the noradrenergic compounds potentiated the discriminative stimulus effects of a relatively low dose of cocaine, indicating that NE receptor stimulation may have an augmenting role in cocaine's discriminative stimulus effects. Moreover, Kleven and Koek [22] also found that stimulation of the β_2-adrenoceptor, but not the β_1-, α_1- or α_2-adrenoceptors, enhanced the discriminative stimulus effects of 2.5 mg/kg cocaine.

4.3 Serotonin

Schama et al. [38] investigated the effects of altered serotonergic tone on cocaine's discriminative stimulus effects. In that study, groups of squirrel monkeys were trained to discriminate intramuscular injections of 0.3 or 1.0 mg/kg cocaine from saline. Compared to monkeys trained to discriminate 1.0 mg/kg cocaine from saline, the nonselective serotonin receptor agonist quipazine produced a greater percent cocaine-lever selection in monkeys trained to discriminate the 0.3 mg/kg cocaine training dose. In addition, the selective serotonin reuptake inhibitor fluoxetine enhanced cocaine's discriminative stimulus effects in the low dose training group, but not in the high dose training group – findings that further demonstrate the importance of training dose in drug discrimination experiments. Last, Schama et al. [38] observed that pretreatment with ketanserin and ritanserin (5-HT_2 receptor antagonists) attenuated cocaine's discriminative stimulus effects in the low dose and high dose group, respectively, indicating a modulatory role of serotonin in mediating cocaine's discriminative stimulus effects. In a rodent drug discrimination experiment, Filip et al. [39] reported that pretreatment with the 5-HT_{2A} antagonist SR 46349B (0.5–1 mg/kg) produced a rightward shift in the dose–response curve for cocaine in rats trained to discriminate 10 mg/kg cocaine from saline, indicating that stimulation of 5-HT_{2A} receptors modulates the discriminative stimulus effects of cocaine. In that study, pretreatment with a 5-HT_{2B} (SB 204741; 1–3 mg/kg) or a 5-HT_{2C} antagonist (SDZ SER-082; 0.5–1 mg/kg) produced no change or a leftward shift (i.e., enhanced the discriminative stimulus effects) in the cocaine dose–response curve, respectively. Finally, as previously mentioned, cocaine does possess low binding affinity for serotonin 5-HT_3 receptors as measured in vitro [10]. As

such, Paris and Cunningham [40] investigated the role of 5-HT_3 receptors in mediating cocaine's discriminative stimulus effects. In that study, 5-HT_3 antagonists ICS 205930 (2–24 mg/kg) and MDL 72222 (2–16 mg/kg) both failed to substitute for cocaine's discriminative stimulus effects in rats trained to discriminate 10 mg/kg from saline. In addition, pretreatment with the 5-HT_3 antagonists failed to block cocaine's (5 mg/kg) discriminative stimulus effects. Thus, the results reported by Paris and Cunningham [40] indicate that altered activity of the 5-HT_3 receptor does not affect cocaine's discriminative stimulus effects, despite cocaine possessing pharmacologically relevant binding affinity at this receptor. In sum, these findings would seem to indicate subtle regulatory effects of altered serotonergic tone [38] and serotonin receptor stimulation [39] on cocaine's discriminative stimulus effects (for review, [41]), but certainly do not challenge the primacy of dopaminergic mechanisms in mediating cocaine-like interoceptive effects.

4.4 Non-monoaminergic Receptors

As mentioned previously, cocaine also possesses pharmacologically relevant binding affinity at muscarinic M_1 and M_2 receptors [11] and σ-receptors [12]. Tanda and Katz [42] investigated the effects of muscarinic M_1 receptor blockade on cocaine's discriminative stimulus effects. In that study, the M_1 antagonists telenzepine and trihexyphenidyl failed to substitute for cocaine's discriminative stimulus effects in rats trained to discriminate 10 mg/kg cocaine from saline; however, when the drugs were injected prior to cocaine administration, the antagonists enhanced cocaine's discriminative stimulus effects (i.e., produced a leftward shift in the cocaine dose–response curve) demonstrating that M_1 antagonism can increase the saliency of cocaine's interoceptive cue. In a recent study, Hiranita et al. [43] observed that the σ-receptor agonists PRE-084 and DTG (delivered at different pretreatment times via intraperitoneal, subcutaneous, or intravenous administration routes) both produced low percent cocaine-lever selection in rats trained to discriminate 10 mg/kg cocaine from saline. As above, these findings would seem to imply regulatory roles for some non-dopaminergic receptors in the discriminative stimulus effects of cocaine.

5 Monoamine Transporter Substrate/Releaser: MDMA

5.1 Stimulant: Hallucinogen Continuum

3,4-Methylenedioxymethamphetamine (MDMA) is a ring-substituted phenethylamine containing a chiral center which allows for stereoisomerism (see discussion on stereochemistry below). Although MDMA is abused (as a primary psychoactive

ingredient in ecstasy or Molly) in its racemic form, it is informative to also consider the discriminative stimulus effects of its component enantiomers. Like cocaine, MDMA is a nonselective ligand at DAT, NET, and SERT, but unlike cocaine it functions as a substrate/releaser at these transporters (e.g., [44]). Although MDMA shares structural similarities to other phenethylamine derivatives (e.g., amphetamine; 2,4-dimethoxy-4-methylamphetamine [DOM] and bupropion [Wellbutrin®]), its discriminative stimulus effects are complex given its stereochemistry. Before discussing MDMA's stereochemical profile, it should be noted that MDMA produces multiple interoceptive effects that can be broadly categorized as stimulant- and hallucinogen-like. For example, in rats trained to discriminate 1.75 mg/kg MDMA from saline, Oberlander and Nichols [45] reported that *S*(+)-amphetamine produced full substitution for MDMA's discriminative stimulus effects in less than half of the subjects, while the hallucinogen lysergic acid diethylamide (LSD) produced 78% MDMA-lever responding. In addition, the hallucinogen DOM produced 56% MDMA-lever responding. These findings indicate that MDMA possesses a complex substitution profile that can be characterized as stimulant-like (*S*(+)-amphetamine, via DA-releasing effects) and hallucinogen-like (LSD and DOM, via agonist effects at serotonergic receptors). It is worth noting that rats can discriminate MDMA from *d*-amphetamine using a three-choice discrimination procedure (e.g., [46]), indicating that the discriminative stimulus effects of these compounds are similar, but nevertheless dissociable.

Broadbear et al. [47] trained female and male Sprague-Dawley rats to discriminate 1.5 mg/kg MDMA from 1.0 mg/mg *dl*-amphetamine and saline using a three-choice discrimination procedure. Any difference between males and females in acquisition of drug stimulus control was not reported. MDMA (0.38–1.5 mg/kg) and *dl*-amphetamine (0.25–1 mg/kg) equipotently substituted in males and females, although females were more sensitive to 0.75 mg/kg MDMA than males. Last, females were less sensitive than males to the rate-decreasing effects of *dl*-amphetamine. Overall, the report by Broadbear et al. [47] demonstrated that males and females do not display major differences in their ability to discriminate MDMA from *dl*-amphetamine and saline, as evidenced by comparable substitution profiles determined with these compounds.

In humans, MDMA produces increases in arousal, positive mood, vigor, and somaesthesia (user feels separate from their body) [48]. In addition, in humans trained to discriminate 20 mg *d*-amphetamine or *meta*-chlorophenylpiperazine (*m*CPP, a nonselective serotonergic agonist) from placebo, half of the subjects reported that MDMA felt similar to *d*-amphetamine and the other half reported effects similar to *m*CPP [48]. These findings are qualitatively similar to the previously described experiments conducted in rodents, and further buttress experimental observations that MDMA's discriminative stimulus effects comprises both dopaminergic and serotonergic components.

5.2 Stereoisomerism

As mentioned, MDMA contains a chiral center and thus exists as a pair of stereoisomers. Previous drug discrimination research has demonstrated that the discriminative stimulus effects of the stereoisomers (*S*(+)- and *R*(−)-isomers) produce different substitution profiles. In the first study to examine the stereoisomers of MDMA in mice using drug discrimination procedures, Murnane et al. [49] reported that the psychostimulants *d*-amphetamine and cocaine produced greater or more potent percent drug-appropriate responding in mice trained to discriminate 1.5 mg/kg of the *S*(+) isomer compared to mice trained to discriminate 1.5 mg/kg of the *R*(−)-isomer. In contrast, the hallucinogens 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) and *N*,*N*-dipropryltryptamine (DPT) produced greater or more potent percent drug-appropriate responding in the *R*(−)-isomer-trained mice than the *S*(+)-isomer-trained mice. These findings indicate that the discriminative stimulus effects of the *S*(+)-isomer of MDMA are more stimulant-like, while the *R*(−)-isomer of MDMA is more hallucinogenic-like [49].

5.3 Pharmacokinetic Considerations

An important determinant of a drug's discriminative stimulus effects is its pharmacokinetic and metabolic profile. Indeed, the onset of a drug's effects, its duration of action, and the potential influence of behaviorally active metabolites can affect a subject's performance in drug discrimination experiments. Fortunately, drug discrimination procedures permit time course analysis of a drug's discriminative stimulus effects, and if knowledge exists about a drug's metabolic disposition, the potential role of metabolites can be examined as well. Fantegrossi et al. [50] investigated the onset and duration of discriminative stimulus effects of MDMA and its enantiomers in mice. In this study, three different groups of mice were trained to discriminate 3.0 mg/kg racemic MDMA, 1.5 mg/kg *S*(+)-MDMA, or 1.5 mg/kg *R*(−)-MDMA from saline. Substitution tests were conducted with the three forms of MDMA in each training group. The results of the substitution tests revealed that racemic MDMA and the *S*(+) isomer produced full substitution in the 3.0 mg/kg racemic MDMA-trained mice, but the *R*(−) isomer failed to produce >20% racemic MDMA-appropriate responding. Results of the time course analysis revealed that 3.0 mg/kg racemic MDMA and 1.5 mg/kg *S*(+)-MDMA (IP) produced relatively rapid (<20 min) onset of discriminative stimulus effects and mice responded on the drug-paired lever for 60 min post-injection. In contrast, 1.5 mg/kg *R*(−)-MDMA reached peak discriminative stimulus effects at 20 min and responding on the drug-paired lever decreased at 40 min post-injection. These findings indicate that MDMA's discriminative stimulus effects may be primarily driven by the pharmacological effects of *S*(+)-MDMA, and to a lesser extent, *R*(−)-MDMA. Alternatively, the component enantiomers may determine different "phases" of the

discriminative stimulus effects of racemic MDMA, with primary contributions of the *S*(+)-enantiomer at early time points, and a role for the *R*(−)-enantiomer emerging some time after administration. Furthermore, although the pharmacokinetic and metabolic profile of MDMA as observed in mice differs from observations conducted in humans [50, 51], the drug discrimination assay is useful for investigating the complex role of pharmacokinetic factors in determining a drug's discriminative stimulus effects.

5.4 Discriminative Stimulus Effects of MDMA

In addition to the discriminative stimulus effects of MDMA isomers, racemic MDMA produces an interesting, yet imperfectly understood, substitution profile. In rats trained to discriminate 0.5 mg/kg *d*-amphetamine from saline, Harper et al. [52] reported that MDMA produced approximately 50% drug-appropriate responding; however, in rats trained to discriminate 1.5 mg/kg MDMA from saline, *d*-amphetamine failed to produce >20% drug-appropriate responding. These findings indicate an asymmetrical substitution profile of MDMA (that is, MDMA may substitute for the discriminative stimulus effects of a different drug, but the other drug may not substitute for MDMA's discriminative stimulus effects, or vice versa). Other reports also support the asymmetric substitution profile observed with MDMA. Khorana et al. [53] trained groups of rats trained to discriminate 1.5 mg/kg MDMA or 8 mg/kg cocaine from saline. In the 1.5 mg/kg MDMA group, cocaine fully substituted for the MDMA cue. In contrast, MDMA failed to produce >36% cocaine-lever selection in the 8 mg/kg cocaine group. These findings indicate that the discriminative stimulus effects of MDMA depend on the training histories of the subjects (i.e., different training drugs in this example), further underscoring the important principle that discriminative stimulus effects of drugs are not immutable properties of those drugs, but are the result of a complex interaction of biological, environmental, and behavioral variables.

As a final point, it is noteworthy that any drug's discriminative stimulus effects is dose-dependent and, in the case of MDMA, possibly along a serotonergic-dopaminergic continuum as the dose increases. For example, Harper et al. [54] trained rats to discriminate 0.5 mg/kg *d*-amphetamine or 1.5 mg/kg MDMA from saline using a three-choice discrimination procedure. In that study, intermediate doses of MDMA (1.0 and 1.5 mg/kg) produced responding primarily on the MDMA-paired lever, but at larger doses of MDMA (3.0 and 4.5 mg/kg), subjects shifted responses to the 0.5 mg/kg *d*-amphetamine-paired lever. These results indicate that as the dose of MDMA increases, the discriminative stimulus effects of MDMA resemble that of a prototypical DA releaser. Using a three-choice discrimination procedure, Goodwin and Baker [46] trained rats to discriminate 1.5 mg/kg MDMA or 1 mg/kg *d*-amphetamine from saline. In that study, substitution tests with *d*-amphetamine produced equivalent percent responding on the *d*-amphetamine, MDMA, and saline levers at 0.25 mg/kg *d*-amphetamine; however,

rats shifted responding to the *d*-amphetamine lever following 0.5 and 1 mg/kg *d*-amphetamine injections. Substitution tests with cocaine produced little responding on the MDMA lever, but instead elicited only dose-dependent responding on the *d*-amphetamine lever. The hallucinogens LSD and DOM produced responding on the saline lever at low doses, but rats shifted their responding to the MDMA lever following larger doses of LSD, and saline and MDMA lever selection became equivalent around 40–50% at larger doses of DOM. Finally, rats tested with the serotonin releaser fenfluramine shifted responding to the MDMA lever at larger doses, and pretreatment with the 5-HT_2 receptor antagonist pirenperone (0.16–0.64 mg/kg) (for more information on pirenperone, [55]) reduced MDMA-lever selection when administered in combination with 1.5 mg/kg MDMA. These findings indicate that drugs with agonist effects at serotonergic receptors (LSD and DOM) elicit interoceptive effects similar to those of MDMA, while drugs with antagonist effects at serotonergic receptors (pirenperone) attenuate the discriminative stimulus effects of MDMA.

To conclude this section, it should be mentioned that a previous report by Harvey and Baker [56] included substitution tests with two synthetic cathinones (MDPV [0.125–3 mg/kg], mephedrone [0.25–2 mg/kg]) in groups of rats trained to discriminate 1.5 mg/kg MDMA or 1.5 mg/kg MDMA +0.5 mg/kg *d*-amphetamine from saline. The mixture group was presumably included to add a more salient dopaminergic component to the 1.5 mg/kg MDMA cue. MDPV produced full substitution in the 1.5 mg/kg MDMA +0.5 *d*-amphetamine group, but only produced partial substitution in the 1.5 mg/kg MDMA group, indicating that the DA-releasing effects of *d*-amphetamine was necessary to generalize to MDPV's interoceptive effects. Mephedrone equipotently produced full substitution in both training groups, indicating that mephedrone's cue involves dopaminergic, serotonergic, and possibly noradrenergic components.

6 Monoamine Transporter Substrate/Releaser: *d*-Amphetamine and Methamphetamine

6.1 d-*Amphetamine: Prototypical DA Releaser*

The phenethylamine derivative *d*-amphetamine produces increases in cytoplasmic DA concentrations through pharmacological effects at intraterminal vesicles, DAT, and monoamine oxidase (a catalytic enzyme) (see [57]). In addition to increasing DA concentrations in extracellular space, *d*-amphetamine alters levels of other neurotransmitters, such as norepinephrine, serotonin, and acetylcholine [57]. Despite these other pharmacological effects, *d*-amphetamine is considered a prototypical DA releaser and is used extensively in drug discrimination research to evaluate the role of increased DA neurotransmission in a novel drug's discriminative stimulus effects.

Smith et al. [58] demonstrated that activation of dopamine D_1-like and D_2-like receptors mediates *d*-amphetamine's discriminative stimulus effects. In rats trained to discriminate 1 mg/kg *d*-amphetamine from saline, the D_2-like receptor agonist quinpirole produced complete generalization; however, the D_1-like receptor agonist SKF 38392 produced no substitution. Pretreatment with 0.2 or 0.5 mg/kg quinpirole, followed by an injection of 0.3 mg/kg *d*-amphetamine, produced complete generalization, whereas 0.3 mg/kg *d*-amphetamine delivered alone produced only partial substitution [58]. Although SKF 38392 produced no substitution when administered alone, pretreatment with doses of SKF 38392 followed by an injection of 0.3 mg/kg *d*-amphetamine produced complete generalization. These findings indicate that the dopamine D_1-like and D_2-like receptors are involved in mediating *d*-amphetamine's discriminative stimulus effects: D_2-like receptor stimulation produces discriminative stimulus effects that are similar to *d*-amphetamine's discriminative stimulus effects, and D_1-like receptor stimulation can potentiate *d*-amphetamine's discriminative stimulus effects. In human participants, Vansickel et al. [59] compiled the results of six studies performed in their laboratory to assess whether women discriminated 15 mg *d*-amphetamine differently than men. Substitution tests were performed with *d*-amphetamine (2.5–15 mg). There were no differences in acquisition of drug stimulus control or *d*-amphetamine substitution between males and females; however, male participants rated 10 and 15 mg *d*-amphetamine as producing a significantly greater high than in females, and males also reported significantly less nausea at 2.5 and 5 mg *d*-amphetamine compared to females. It should be emphasized that despite these qualitative differences between males and females after exposure to *d*-amphetamine, their discrimination performances were similar. As such, there may be qualitative differences between males and females in experiencing *d*-amphetamine's interoceptive cue (i.e., information that can be gathered by verbal reports), but no difference in males' and females' *ability to detect* said cues. Future drug discrimination experiments with human participants will be useful for further identifying the subjective qualities of drugs' interoceptive effects under experimental conditions.

The previous summary of cocaine's discriminative stimulus effects suggests that cocaine produces discriminative stimulus effects that are similar to *d*-amphetamine's discriminative stimulus effects (i.e., both drugs produce increases in extracellular DA concentrations), and vice versa. Indeed, both *d*-amphetamine [23, 60] and methamphetamine ([23]; see below for discussion of methamphetamine) produce complete generalization in rats trained to discriminate 10 mg/kg cocaine from saline.

6.2 *Methamphetamine: Prototypical DA Releaser*

Similar to *d*-amphetamine, methamphetamine disrupts vesicular dopamine storage (e.g., [61]) and produces regional increases in DA content (e.g., [62]), although methamphetamine also alters serotonergic neurotransmission to a greater extent

than amphetamine [62]. In rats trained to discriminate 1 mg/kg methamphetamine, Munzar et al. [63] reported that phentermine (an amphetamine analog) produced complete generalization. In addition, phentermine administered in combination with 1 mg/kg fenfluramine produced a rightward shift in phentermine's dose–response curve, indicating that the 5-HT-releasing effects of fenfluramine decreased the potency of phentermine's discriminative stimulus effects. In a human drug discrimination experiment, Lamb and Henningfield [64] trained human volunteers to discriminate 30 mg *d*-amphetamine from placebo. Substitution tests were conducted with *d*-amphetamine, methamphetamine, and the μ-opioid agonist hydromorphone. Results revealed that *d*-amphetamine and methamphetamine produced full substitution for *d*-amphetamine's discriminative stimulus effects; however, hydromorphone failed to produce >30% drug-appropriate responding indicating that the interoceptive effects of hydromorphone were dissimilar from the effects produced by *d*-amphetamine. It should be noted that the drug discrimination assay is pharmacologically selective; that is, if participants are trained to discriminate a compound that increases intraterminal release of dopamine (e.g., *d*-amphetamine), then a compound with binding affinity to the μ-opioid receptor (e.g., hydromorphone) is unlikely to produce complete generalization. Indeed, many drug discrimination experiments include a substitution test compound with a different pharmacological mechanism of action to serve as a negative control (i.e., the researchers predict that the drug will engender low percent drug-lever selection).

Norepinephrine has also been studied as a potential modulator of methamphetamine's discriminative stimulus effects. In rats trained to discriminate 1 mg/kg methamphetamine from saline, the selective NET inhibitors desipramine and nisoxetine did not substitute for methamphetamine when administered alone, but each compound significantly shifted the methamphetamine dose–response curve to the left when administered as pretreatments [65] demonstrating that they potentiated methamphetamine's discriminative stimulus. Interestingly, and in apparent contrast with cocaine, neither the β-adrenoceptor agonist isoproterenol nor the antagonist propranolol generalized to methamphetamine when given alone nor altered the discriminative stimulus effects of methamphetamine when administered in combination [65]. No systematic or dose-related effects of the α-adrenoceptor agonists methoxamine (α_1) and clonidine (α_2) or the α-adrenoceptor antagonists prazosin (α_1) or yohimbine (α_2) were apparent when substituted for methamphetamine or when administered in combination with methamphetamine, although the α_2 ligands tended to produce larger magnitude effects in comparison with the α_1 ligands.

As previously noted with cocaine, 5-HT receptor subtypes appear to modulate the discriminative stimulus effects of methamphetamine in the rat. One notable study directly compared the effects of the hallucinogenic 5-HT_2 agonist DOI on cocaine-like interoceptive effects occasioned by either cocaine or methamphetamine in rats trained to discriminate 10 mg/kg cocaine from saline [66]. As expected, methamphetamine fully substituted for cocaine in these subjects, but while pretreatment with DOI did not alter the dose-effect curve for cocaine, it

dramatically potentiated the cocaine-like discriminative stimulus effects of methamphetamine, as evidenced by a twofold shift in the methamphetamine dose-effect curve [66]. These data indicate that 5-HT_2 receptor activation is involved in psychostimulant discriminative stimulus effects, and may be particularly salient in the context of the interoceptive effects of methamphetamine.

7 Conclusion

The discriminative stimulus effects of psychostimulants have been well characterized using drug discrimination procedures. Regarding the psychostimulants presented in this chapter, monoaminergic neurotransmission largely mediates their discriminative stimulus effects, with dopamine being the most salient neurotransmitter in this regard (see Table 1). Moreover, comparable substitution profiles of psychostimulants are observed across species (e.g., mice, rats, nonhuman primates, and humans), indicating that drug discrimination procedures produce reliable observations of their discriminative stimulus effects. Although there is a paucity of drug discrimination experiments that directly compare the discrimination performance of females to males, there appears to be little difference in ability to discriminate interoceptive cues of psychostimulants as a function of sex in rodents. Instead, it is possible that the qualitative nature of a drug's interoceptive cue (i.e., the information that can be provided through verbal report in humans) differs between sexes. Future drug discrimination experiments in humans who possess a verbal repertoire are necessary to elucidate further any differences in qualitative aspects of a drug's discriminative cue.

In addition to providing translational value, drug discrimination procedures also permit analysis of drug stereochemistry, pharmacokinetics, and metabolic interactions. From this chapter, readers may glean the complexity that is involved in determining a drug's discriminative stimulus effects. Indeed, the discriminative stimulus effects of psychoactive substances vary with an innumerable number of biological, environmental, and behavioral factors, many of which were not addressed here. Nevertheless, the drug discrimination assay, in its most basic form, reveals pharmacological effects that occur within the central nervous system in species that display little to no verbal communication. We consider this an achievement in scientific research in general, and we submit that the drug discrimination approach is among the most useful in vivo analyses available to behavioral pharmacology.

Table 1 Protein mechanisms that produce full substitution, weaken, or enhance the interoceptive effects of psychostimulants

Psychostimulant training drug	Mechanisms producing full substitution	Mechanisms that weaken interoceptive effects	Mechanisms that enhance interoceptive effects	References/ reviews
Cocaine	– Substrates/ releasers at DAT – Reuptake inhibitors at DAT – D_2 receptor agonists – D_3 receptor agonists – β_2-adrenoceptor antagonist	– D_2 receptor antagonism – D_1 receptor antagonism – Nonselective 5-HT_2 receptor antagonists – 5-HT_{2A} receptor antagonist	– Reuptake inhibitors at DAT – β_2-adrenoceptor antagonism – Selective reuptake inhibitors at SERT (SSRIs) – 5-HT_2c antagonists – Muscarinic M_1 receptor antagonism	[13–20, 23, 24, 26–29, 37–39, 41, 42]
MDMA (racemic and stereoisomers included)	– Substrates/ releasers at monoamine transporters – Reuptake inhibitors at monoamine transporters – 5-$HT_{1A/2A}$ agonists – 5-$HT_{1B/2C}$ agonists	– 5-HT_2 receptor antagonists – 5-HT_3 receptor antagonists – 5-HT depletion – D_1 receptor antagonists – D_2 receptor antagonists (time-dependent effect occurs 105 min post-injection of MDMA)	– 5-HT releaser (following neurotoxic regimen)	[45, 46, 49, 56, 67–73]
d-Amphetamine and Methamphetamine	– DA releasers – D_2 receptor agonists	– DA releasers +5-HT releasers (5-HT releasers have weakening effect)	– D_1 receptor agonists – D_2 receptor agonists – NET inhibitors – 5-HT_2 agonist (shifted methamphetamine curve to right in cocaine-trained rats)	[58, 63, 65, 66]

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Discriminative Stimulus Properties of *S*(−)-Nicotine: "A Drug for All Seasons"

John A. Rosecrans and Richard Young

Abstract *S*(−)-Nicotine is the major pharmacologically active substance in tobacco and can function as an effective discriminative stimulus in both experimental animals and humans. In this model, subjects must detect and communicate the nicotine drug state versus the non-drug state. This review describes the usefulness of the procedure to study nicotine, presents a general overview of the model, and provides some relevant methodological details for the establishment of this drug as a stimulus. Once established, the (−)-nicotine stimulus can be characterized for dose response and time course effects. Moreover, tests can be conducted to determine the similarity of effects produced by test drugs to those produced by the training dose of nicotine. Such tests have shown that the stimulus effects of nicotine are stereoselective [*S*(−)-nicotine >*R*(+)-nicotine] and that other "natural" tobacco alkaloids and (−)-nicotine metabolites can produce (−)-nicotine-like effects, but these drugs are much less potent than (−)-nicotine. Stimulus antagonism tests with mecamylamine and DHβE (dihydro-β-erythroidine) indicate that the (−)-nicotine stimulus is mediated via α4β2 nicotinic acetylcholine receptors (nAChRs) in brain; dopamine systems also are likely involved. Individuals who try to cease their use of

Sadly, **Dr. John A. Rosecrans** passed away during the writing of this chapter. John inspired both students and colleagues with his keen interest and enthusiasm in matters related to biomedical research and, especially, nicotine. Most of all, John will be remembered and missed for his friendship. The editors (J. H. Porter and A. J. Prus) of the book (*The Behavioural Neuroscience of Drug Discrimination*) this chapter is published in would also like to note that the book is dedicated to Dr. John A. Rosecrans.

J.A. Rosecrans
Department of Pharmacology and Toxicology, Virginia Commonwealth University, 410 North 12th Street, P.O. Box 980613, Richmond, VA 23298-0613, USA

R. Young (✉)
Department of Medicinal Chemistry, Virginia Commonwealth University, 800 East Leigh Street, P.O. Box 980540, Richmond, VA 23219-0540, USA
e-mail: ryoung@vcu.edu

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nicotine-based products are often unsuccessful. Bupropion (Zyban®) and varenicline (Chantix®) may be somewhat effective as anti-smoking medications because they probably produce stimulus effects that serve as suitable substitutes for (−)-nicotine in the individual who is motivated to quit smoking. Finally, it is proposed that future drug discrimination studies should apply the model to the issue of maintenance of abstinence from (−)-nicotine-based products.

Keywords Anabasine • Anatabine • Cotinine • Drug abuse • Drug discrimination • Lobeline • Methyllycaconitine • Nicotine • Nornicotine • Stereoisomers

Contents

1 Introduction

S(−)-Nicotine is one of the oldest and most widely used psychoactive drugs. Historically, (−)-nicotine ingestion, through tobacco smoking, has been traced to the Mayan civilization in Mexico (circa 600 A.D.). In the pre-Columbian Americas, it was smoked in pipes, chewed, and/or insufflated by itself or in combination with hallucinogenic snuffs (e.g., [1, 2]). Botanically, the tobacco plant belongs to the nightshade family *Solanaceae* and, therefore, is related to tomato and potato plants as well as to "deadly nightshade" (*Atropa belladonna*), from which belladonna (i.e., tropane alkaloids atropine, scopolamine, and hyoscyamine) is derived. Tobacco also belongs to the genus *Nicotiana*, named for Jean Nicot, French ambassador to

Portugal in the mid-sixteenth century. It was Nicot who first sent tobacco to the king of France. From France, its use spread throughout Europe. A South American species, *N. tabacum*, is the source for most of today's commercially marketed tobacco products (e.g., [3–5]).

Chemically, nicotine (1-methyl-2-(3-pyridyl)pyrrolidine; Fig. 1) is a tertiary amine composed of pyridine and pyrrolidine rings whose molecular structure was first proposed by Pinner [6] and confirmed by Pictet and Crepieux [7] and Spath and Bretschneider [8]. Moreover, nicotine has one chiral center (at carbon 2 of the pyrrolidine moiety) and natural nicotine, as constituted in tobacco, has a levorotatory [i.e., (−)] rotation (also called (−)-nicotine or *l*-nicotine). Most importantly, however, (−)-nicotine has the (*S*)-configuration, which provides information about the chemical structure of (−)-nicotine in three-dimensional space and how it may interact with receptors [9].

(−)-Nicotine-based products can generally be divided into two types: smoked tobacco (cigarette/cigar/pipe and hookah smoking of tobacco) and smokeless tobacco (chewing tobacco, snuff, and snus). Recently, however, electronic cigarettes (E-cigarette or E-cig) that produce (−)-nicotine in vaporized form have appeared in the marketplace. An E-cig is a battery-powered vaporizer that is thought to produce a similar sensation to tobacco smoking (a.k.a. "vaping"). The device employs a heating element that atomizes a liquid solution known as e-liquid, which usually contains a mixture of propylene glycol, glycerin, (−)-nicotine, and flavorings. All of these products contain (−)-nicotine but their use can vary significantly from person to person and product to product. For many tobacco users, continued nicotine consumption results in dependence (compulsive nicotine seeking and use), even at the risk of negative health consequences. Smokers, for example, may become physically addicted to (−)-nicotine and link smoking with many social- and work-related activities, which produce difficulties if the individual desires to cease smoking. Furthermore, if (−)-nicotine levels in the body are changed, smokers tend to compensate to reach their "comfort" level of drug by smoking more or less if the levels of nicotine are reduced [e.g., by administration of mecamylamine, a noncompetitive nicotinic receptor antagonist (see "mecamylamine" below)] or are increased [e.g., administration of exogenous (−)-nicotine], respectively. Moreover, smokers can "titrate" the level of (−)-nicotine in their system with adjustments in the number of puffs on a cigarette, duration of puffs, inter-puff intervals, and/or number of cigarettes smoked (e.g., [10]).

When a nicotine product is smoked, chewed, or inhaled, it is readily absorbed into the bloodstream and penetrates the blood–brain barrier to produce central effects. In addition, its peripheral actions include effects on the autonomic ganglia,

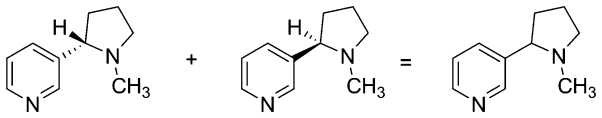

Fig. 1 Structures of *S*(−)-nicotine (*left*), *R*(+)-nicotine (*center*) and racemic nicotine (*right*)

adrenal medulla, and neuromuscular junction. It is important to note that (−)-nicotine acts as a stimulant at these sites only when administered at relatively low doses. When higher doses of the drug are administered, membranes are depolarized and maintained in the depolarized state for an extended period of time; i.e., blockade of nicotinic cholinergic (nACh) signals. This biphasic action of nicotine (stimulation followed by blockade of transmission due to a maintained depolarization) can complicate the formation of clear conclusions of its pharmacological actions. Consequently, (−)-nicotine often exhibits a "narrow-window" or steep dose-effect function between doses (or concentrations) that produce excitation and doses that exert blockade of biological actions. The interested reader is referred to Matta et al. [11], who have reviewed and compiled recommended doses of (−)-nicotine for in vivo research. In particular, these authors have noted that responses to (−)-nicotine often display a bell-shaped (inverted U-shaped) dose-response profile.

In humans, (−)-nicotine can function as both a "stimulant" and a "sedative." For example, immediately after exposure to nicotine, there is a "stimulant-kick" caused, in part, by its stimulation of the adrenal glands and resultant discharge of epinephrine (adrenaline). The release of epinephrine stimulates the body and causes a sudden release of glucose as well as an increase in blood pressure, respiration, and heart rate. Nicotine also suppresses insulin output from the pancreas, which indicates that smokers are usually hyperglycemic (higher blood sugar level). Centrally, (−)-nicotine has affinity for all brain nAChR subtypes, but binds preferentially and with high affinity to α4β2 nAChRs (e.g., [12, 13]). Moreover, (−)-nicotine (indirectly) can produce a release of dopamine in brain regions that are thought to control pleasure and motivation; dopamine is thought to underlie the pleasurable sensations experienced by smokers (e.g., [14, 15] but see [16]). In addition, nicotine also can exert a sedative effect, depending on the smoker's level of arousal and administered dose of nicotine. Thus, (−)-nicotine seems to produce a unique combination of effects: when stimulation is needed, smokers may perceive the "smoke as a stimulant," and when they feel anxious and desire relief, they may perceive the "smoke as a tranquilizer." In this regard, the first author of this review has often referred to the human appeal for nicotine as "a drug for all seasons."

The dual effects of (−)-nicotine also can be seen in animal behavior. For example, in rodents, administration of low doses of nicotine produced increased motor activity whereas high doses produced decreased motor activity (e.g., [17, 18]). Moreover, the effects of nicotine on motor activity of animals can be dependent on pre-drug activity levels. That is, nicotine caused decreased activity of rodents that had a high pre-drug level of activity and produced increased activity of animals that had a low pre-drug level of activity. Also, pharmacological effects of nicotine have been observed to be contingent on whether subjects were pre-exposed to the behavioral paradigm under investigation. Lastly, different strains and gender of rodents have been shown to interact differentially in these aforementioned effects (e.g., [19–21]). Taken together, the effects of nicotine seem to be markedly dependent on the dose of (−)-nicotine as well as subjects' pre-drug level of activity, pre-exposure (i.e., level of tolerance) to nicotine and familiarity with the behavioral

paradigm (e.g., [22]; reviewed in [23]). As such, these studies have provided important data as to how the acute effects of nicotine can affect the behavior of animals and, by extension, of humans. On the other hand, these assays of the acute effects of (−)-nicotine tend to engender much variability in results and this has led to searches of animal models in which dependent measures are more stable.

2 Drug Discrimination

A complete review of the drug discrimination literature of (−)-nicotine is beyond the scope of this review. Rather, the focus here is a description of the usefulness of the drug discrimination procedure to study (−)-nicotine, methodological issues and procedures, stereochemical aspects of nicotine, stimulus effects of other tobacco alkaloids and/or (−)-nicotine metabolites, nicotinic acetylcholine receptor (nAChR) mechanisms of action, and current pharmacotherapy for cessation of (−)-nicotine ingestion.

2.1 Why Use Drug Discrimination (DD) Procedures to Study S(−)-Nicotine?

An early study in humans by Johnston [24] demonstrated that the injection of (−)-nicotine was perceived as "pleasant" to smokers and "unpleasant" to nonsmokers. In fact, this study may have been the first scientific demonstration that nicotine has "appeal" to smokers that is not readily apparent to nonsmokers. A pivotal reason that nicotine has appeal to smokers, but not to nonsmokers, is that tolerance has developed to the unpleasant acute effects produced by nicotine or other tobacco constituents that are experienced by neophyte smokers; nausea and/or vomiting, dizziness, sweating, pallor, headache, and weakness (e.g., [25]). The inexperienced smoker cannot usually abide the amount of (−)-nicotine present in a single cigarette, but after sufficient experience with their consumption may be able to smoke many cigarettes over a relatively short period of time without the experiences of these adverse effects. Thus, the acute effects of (−)-nicotine may include more and/or different pharmacological actions than the chronic effects of (−)-nicotine. In fact, acquired tolerance to these adverse effects of nicotine probably exerts an important role in the acquisition and maintenance of dependence and consequent health problems that are linked to the use of tobacco products (e.g., [26]).

Basic research of the effects of (−)-nicotine on biological/behavioral variables has mainly employed acute nicotine treatment. However, human users of nicotine products are exposed to the substance chronically. Similarly, subjects in drug discrimination studies are exposed to training drug [e.g. (−)-nicotine] chronically. Thus, discrepancies in results between acute studies and chronic investigations of

nicotine may be due to differences in responses from subjects who have different sensitivities to nicotine. Moreover, subjects' neural adaptations that result from repeated exposure to nicotine, such as with human smokers or participants in drug discrimination procedures, are very unlikely to be seen in subjects exposed to acute administration of nicotine (e.g., [27–29]). Taken together, drug discrimination procedures appear to simulate, to a reasonable degree, human involvement with (−)-nicotine over time (e.g., [30]). Moreover, the drug discrimination paradigm is one of only a few preclinical assays to have a counterpart procedure for humans (e.g., [31–33]).

Drug discrimination procedures are dependent on the ability of a subject to detect a specific drug state, which is similar to a human report of the subjective effects produced by a drug. This approach does not focus on the behavioral effects of a drug, but instead, is used to study subjects' internal reactions or "perceptions" of the drug effect(s). *In other words, the paradigm allows subjects to identify the effects of (−)-nicotine rather than being a procedure that studies the excitatory or disruptive effects of (−)-nicotine on behavior*. Thus, the DD procedure is not measuring the disruptive or other acute pharmacological effects (e.g., stimulation) of nicotine, but only the ability of an animal to detect the "state" produced by nicotine after chronic administration. As such, animals typically become behaviorally tolerant to the disruptive (acute) effects of (−)-nicotine given at the beginning of training so that experimental results are not encumbered by changes in rates of behavior. Importantly, however, tolerance to the stimulus effects of (−)-nicotine does not readily occur, which allows the experimenter to study the effects of nicotine in a repeated- or within-subjects experimental design over an extended period of time (often $\geq$2 years, see [30]). If tolerance did occur, then subjects would no longer be able to demonstrate that they recognize differences in effects between their training dose of nicotine and saline vehicle (control) states.

(−)-Nicotine, like many psychoactive drugs, can exert discriminative control over behavior (for review, see [34]). Historically, the first detailed publication on the stimulus properties of nicotine was reported by Morrison and Stephenson [35], who trained rats to discriminate the effects of 0.4 mg/kg (s.c.) of (−)-nicotine from saline in a two-lever operant conditioning task. Shortly thereafter, Rosecrans and colleagues published a series of studies in rats trained to discriminate (−)-nicotine from saline in both T-maze and two-lever operant tasks [36–41]. These studies firmly established that (−)-nicotine could serve as a centrally mediated discriminative stimulus in rats. Subsequently, other species have been used to establish nicotine as a discriminative stimulus; monkey, mouse, and human (e.g., [32, 33, 42–45]). The rat, however, is most commonly employed. Moreover, results of drug discrimination studies with non-human animal subjects and human research participants have shown a relatively high degree of concordance, which suggests that the DD model may reflect the internal or "subjective" effects of (−)-nicotine in humans (e.g., [32, 46]). The rationale and methods described in these early reports are still relevant today and are recapitulated below (also see [30, 47, 48]).

2.2 Rationale

The discriminative stimulus effects of (−)-nicotine involve procedures designed to assess the effects of this drug to exert control over behavior. In the paradigm, a subject is trained to make differential behavioral responses contingent upon administered treatments. An experimental participant (e.g., rodent) is trained to emit one response (such as pressing one lever in a two-lever operant chamber to obtain a reinforcer) following one treatment (e.g., dose of drug), and another response (that is, pressing the opposite-side lever) following a different treatment [e.g., saline vehicle (non-drug)]. These behavioral responses are highly dependent on the subject being able to detect a specific drug state and are similar to the requirement of a human to report the subjective effects of a given psychoactive drug. In these situations, behavioral responses performed by subjects are under the stimulus control of the administered dose of training drug. In other words, the animals' lever responses represent, or are reflective of, their subjective "experience" under a given treatment.

2.3 Methodology

Early drug discrimination studies of (−)-nicotine used the T-maze procedure in both positively reinforced and escape tasks, whereas later (and current) studies employed the use of two-lever operant chambers. T-maze tasks required subjects (usually rats) to choose between two alleys on each of several trials. In a typical maze experiment, a rat may have been trained to turn to the right-side alley (i.e., designated the drug-side for that rat) to obtain food reward or escape mild electric shock (i.e., consequences) after administration of its dose of training drug, and to turn to the left-side alley (i.e., designated the vehicle-side for that same rat) to receive consequences after injection of vehicle (usually saline). Experimenters considered the animals' first response during the first trial of sessions, before any consequence (e.g., reward or escape), as a reflection of the degree to which animals had learned to select the treatment-appropriate (i.e., correct) response. There were, however, a number of reasons for the decline in the use of the T-maze and the increased use of two-lever operant tasks. T-maze use declined because a consensus of thought among investigators was that (a) higher doses of drug [(−)-nicotine] were needed to train rats in T-maze procedures than in lever tasks and (b) 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 T-maze response was considered, the evaluation of stimulus control was based on a very small sample of responses. In comparison, the two-lever procedure allowed animals to respond at any rate on either lever, and the data could be expressed in terms of % drug [i.e., (−)-nicotine]-appropriate responding.

2.3.1 Initial Shaping of Behavior

Rats (usually) are food-restricted to 80–85% of their growing body-weight and shaped to lever-press for reinforcement (e.g., food pellet or sweet milk) with one lever in the operant chamber. The shaping procedure required subjects to be placed in the experimental chambers and taught to lever-press under a fixed ratio-one (FR-1) schedule of reinforcement, such that every lever-press was rewarded. During this initial exposure to training, rats were trained with only the right- or left-side lever in the operant chamber. Typically, half of the subjects were required to press the left-side lever and the other half the right-side lever to obtain reinforcement. The latter tactic was (is) important because of the finding that rodents may learn to use olfactory cues (hints) that remained on the levers by animals that preceded them [49]. After initial shaping, rats are exposed to at least four additional daily 15 min training sessions on the same lever (right or left), during which saline (1 mL/kg; s.c.) was administered 10 min prior to behavioral training; consequently, correct-lever responding in the non-drug (i.e., saline) state was established.

2.3.2 Training Under Both Drug and Non-drug Conditions

Once rats are shaped to lever-press under the saline condition, each subject was then trained to lever-press for food on the opposite lever (again with only one lever present in the chamber) 10 min after the administration of (−)-nicotine (typically, a training dose was chosen between 0.1 and 0.4 mg/kg, s.c.). As under the saline training condition, subjects are exposed to drug for, at least, four daily 15-min sessions. After subjects are trained to lever-press for food separately under both drug and saline conditions, with only one lever present in the chamber (approximately 8–10 training sessions), both levers are then introduced into the chamber and rats are trained daily for 15 min [10 min after either saline or (−)-nicotine administration]. (−)-Nicotine and saline treatment sessions are introduced with a double alternation design: i.e., 2 days with (−)-nicotine and 2 days with saline. This double alternation schedule is maintained throughout the study. During the training procedure, a specific schedule of reinforcement, typically a fixed ratio-10 (FR-10) or variable interval 15-s (VI-15 s) is introduced gradually in order to provide added control over behavior. In an FR schedule, the performer completes a fixed number of responses in order to obtain reinforcement; for example, on an FR10 schedule, every 10th response is reinforced. In VI schedules, the length of time that elapsed before reinforcement is delivered varies around the mean value specified by the schedule; for example, on a VI 15 s schedule, reinforcement is available, on average, after 15 s has elapsed since the last reinforcement, but may be available as shortly as 2 s later, or not until 60 s has elapsed. The first response after a time interval has elapsed produces reinforcement for the subject. Besides these operant schedules of reinforcement, subjects may learn other schedules of reinforcement or ways to discriminate a specific dose of drug from vehicle (for review, see [34]). In

studies described below, however, rats learned to discriminate (−)-nicotine from saline on an FR or VI schedule of reinforcement in about 3 months, following 20–30 sessions under each treatment condition.

2.3.3 Testing Procedures

Behavioral data are collected during test sessions in which each animal is administered training dose of (−)-nicotine, other doses of (−)-nicotine, saline, or doses of other drugs in stimulus generalization or antagonism tests. Test sessions are conducted in which both levers are either reinforced (a technique used sometimes with FR schedules of reinforcement) or not reinforced (a tactic used with VI schedules of reinforcement). The degree of stimulus control exerted by (−)-nicotine is determined during these test sessions, which are interspersed between specific double alternation training sessions. The subjects' rate of learning to discriminate between (−)-nicotine and saline is easily monitored via these short sessions; sometimes, these sessions are followed by training under the treatment-correct condition. In addition, such tests are usually conducted on "crossover" days, in which the drug condition alternates from nicotine to saline or vice versa. The subjects' discrimination of (−)-nicotine from saline is considered optimal when they perform at least 90–95% of their lever-presses on the *nicotine-correct lever* following their training dose of (−)-nicotine, whereas they perform 0–5% of their lever-presses *on that same lever* after administration of saline. Experimental data are expressed as percent responses on the nicotine-appropriate lever. *Thus, all data are related to the nicotine-based discriminative stimulus.*

2.3.4 Challenge Experiments

The animals' discrimination of (−)-nicotine from saline is generally established within 2–4 months following initial shaping procedures, at which time a variety of experiments can be conducted. Moreover, the discriminative stimulus effects of nicotine are evaluated during both training and test sessions for up to 2 years in most experimental subjects [30, 47]. Consequently, many of the rats utilized in these studies were exposed to a minimum of 250 nicotine and saline training sessions. Once a group of test subjects had reached training criteria, drug challenge experiments termed (a) stimulus generalizations tests can be initiated to determine if other drugs produce the training drug-like response and (b) stimulus antagonism tests can be conducted to determine if substances (in combination with the training drug) can interfere with the animals' recognition of the training drug-like response (see mechanisms of action section below).

2.3.5 Stimulus Generalization

Stimulus generalization tests are used to determine if a training drug stimulus will generalize (i.e., substitute) to other drugs. The rationale of this approach is that an animal trained to discriminate a dose of training drug exhibits stimulus generalization only to drugs that exert a similar stimulus effect (though not necessarily through an identical mechanism of action). It is important to note that results of stimulus generalization tests are interpreted in relation to the dose of training drug-like effects. As such, and for example, a study of novel substances in (−)-nicotine-trained animals reflects the actions of the novel agents to produce "(−)-nicotine-like" stimulus effects. In most studies, stimulus generalization is said to have occurred when animals, after administration of a given dose of challenge drug, perform ≥80% of their responses on the (−)-nicotine-appropriate lever. Where stimulus generalization occurred, an effective dose 50% (ED_{50}) value is calculated and reflects the dose at which animals would be expected to make 50% of their responses on the (−)-nicotine-appropriate lever. Besides complete stimulus generalization, two other types of results can occur: partial generalization and saline-like responding. Partial generalization occurs when animals, after being administered a thorough dose effect test, perform approximately ~40–70% of their responses on the nicotine-appropriate lever. Data of this type are very difficult to interpret. However, partial generalization may occur with a test drug because there are pharmacological effects that are common to both the training drug and the test drug; full generalization may not occur because the overlap of pharmacological effects to achieve full substitution is incomplete (for further discussion, see [34]). Lastly, administration of various doses of test drug may result in ≤20% (−)-nicotine-appropriate responding. This type of result does not necessarily mean that a test drug is inert, but may indicate that the effect of the challenge drug is simply different from that produced by the dose of training drug. That is, the saline-designated lever also serves as a default response for a drug effect that is unlike that of the training drug and, hence, animals perform relatively few responses on the nicotine-appropriate lever. For example, Pratt et al. [50] trained rats to discriminate 0.4 mg/kg of (−)-nicotine from saline and reported that test doses between 0.25 and 4 mg/kg of fenfluramine, an appetite suppressant, produced saline-like responding; i.e. a maximum of ~20% (−)-nicotine-appropriate responding. Such doses of fenfluramine are not inert and indicate quite clearly that the stimulus effects produced by 0.4 mg/kg of (−)-nicotine are different from those produced by fenfluramine. Furthermore, some of the tested doses of fenfluramine have been shown to serve as discriminative stimuli (e.g., [51]; for review, see [34]).

3 Characterization of the Stimulus Effects of (−)-Nicotine

Drug discrimination studies of (−)-nicotine typically begin with an evaluation of the "strength" of the training stimulus and include both dose response and time course tests. Early studies indicated that both the rate of learning and sensitivity to the training drug were observed to be dose related; i.e., rats trained at relatively higher doses of (−)-nicotine learned the discrimination at a more rapid rate and appeared less sensitive to relatively lower doses of (−)-nicotine. After repeated training, however, rats exhibited fewer differences among training doses, but the dose response nature of the discrimination remained the same [30]. For example, drug discrimination learning curves of rats trained at three (−)-nicotine training dose levels (either 0.1, 0.2 or 0.4 mg/kg from saline, s.c.) under VI-15 s, FR-10 or differential reinforcement of low (DRL)-10 s rate of responding schedules of reinforcement were compared for nicotine sensitivity, as measured by ED_{50} doses (Table 1). As can be seen, ED_{50} values were proportional to training doses, but an asymptotic effect occurred above 0.2 mg/kg of (−)-nicotine. Thus, the stimulus effects of 0.2 and 0.4 mg/kg of (−)-nicotine were somewhat equipotent after the drug had exerted discriminative control over the animals' behavior. Table 1 also indicates that separate groups of rats trained to discriminate 0.4 mg/kg of (−)-nicotine from saline under the above FR, VI or DRL schedules of reinforcement displayed essentially equipotent ED_{50} doses of nicotine, which indicated that schedule of reinforcement did not markedly influence the "strength" of the stimulus effects of 0.4 mg/kg of (−)-nicotine [30, 48, 52].

Once a training dose of (−)-nicotine has been established as a discriminative stimulus, tests can be performed to determine its time course of action. Such tests investigate the effects of changing the pre-session injection interval of the training dose of drug and the beginning of a test session. For example, time course studies in the previously mentioned three groups of animals trained under the three doses of (−)-nicotine (under the VI-15 s schedule of reinforcement) were evaluated. The results revealed that percent (−)-nicotine-appropriate responding declined to 50% of its initial effect within 140–160 min after 0.4 mg/kg of nicotine, 100 min after 0.2 mg/kg of (−)-nicotine, and 70 min after 0.1 mg/kg of (−)-nicotine; thus, time course was proportional to dose. A further analysis of these time duration relationships also suggested a link between the appearance/disappearance of (−)-nicotine levels measured in brain areas (telencephalon, diencephalon, and brainstem) and

Table 1 *S*(−)-Nicotine dose response evaluations in rats trained under different schedules of reinforcement

Schedule and (−)-nicotine Training dose (s.c.)	ED_{50} dose mg/kg (95% C.L.)
VI-15 s; 0.1 mg/kg	0.026 (0.009–0.071)
VI-15 s; 0.2 mg/kg	0.079 (0.040–0.161)
VI-15 s; 0.4 mg/kg	0.086 (0.040–0.185)
FR-10; 0.4 mg/kg	0.098 (0.042–0.184)
DRL-10; 0.4 mg/kg	0.093 (0.040–0.215)

Data adapted from Chance et al. [52] and Rosecrans [30]

the time-related stimulus effects of (−)-nicotine [52, 53]. Other studies also have stressed the importance of training dose and pre-session injection intervals in the stimulus properties of (−)-nicotine (e.g., [54, 55]).

4 Nicotine Stereoisomers

Structurally, nicotine is a chiral substance that can exist as one of two stereoisomers: *S*(−)-nicotine or *R*(+)-nicotine (Fig. 1). Optical isomers of biologically active drugs typically display differences in potency, and, on occasion, also can display differences in effect. Comparative studies of the lethality and pharmacology of the optical isomers of nicotine are few, but the limited results are mostly consistent and indicate that the effects of the enantiomers are *stereoselective*: i.e. effects are qualitatively similar, but *S*(−)-nicotine is more potent than *R*(+)-nicotine (e.g., [56]). However, and unfortunately, a review of the literature failed to find even one study that directly compared the effects of racemic nicotine to effects of its stereoisomers in the same assay.

4.1 Lethality

S(−)- and *R*(+)-nicotine were reported to be equally toxic after i.p. administration in rats and guinea pigs. However, when guinea pigs were injected s.c., *S*(−)-nicotine was twice as lethal as *R*(+)-nicotine [7, 57]. In comparison, *S*(−)-nicotine was shown to be 7 times more toxic than *R*(+)-nicotine in rats injected intravenously [58]. In other studies, *S*(−)-nicotine was reported to be as toxic as, or slightly more toxic than, (±)-nicotine when administered intraperitoneally in rats, intravenously in rabbits, and intraperitoneally or intravenously in cats [59, 60]. In addition, the former study claimed that synergism resulted when the levorotatory and racemic forms of nicotine were mixed in certain proportions; however, few animals/group were used and no statistics were calculated to support the claim. Also worthy of note is that the lethality of *S*(−)-nicotine is highly dependent upon species [61]. For example, the oral (p.o.) lethal dose 50% (LD_{50}) of (−)-nicotine in mouse ($LD_{50} = 3.3$ mg/kg) is approximately 3 times more potent than that in dog ($LD_{50} = 9.2$ mg/kg) and over 15 times more potent than that in rat ($LD_{50} = 50$ mg/kg). Thus, mice seem to be particularly sensitive to the toxic effects of (−)-nicotine.

4.2 Pharmacology

The first study to examine the behavioral pharmacology of the optical isomers of nicotine compared their effects in a conditioned avoidance task in rats, a preclinical

test that is sometimes used to assess antipsychotic-like effects. Both enantiomers blocked the rats' conditioned avoidance response and *S*(−)-nicotine was 7 times more potent than *R*(+)-nicotine [62]. Studies of drug discrimination with *S*(−)-nicotine as training stimulus have consistently reported that *S*(−)-nicotine is more potent than *R*(+)-nicotine. For example, the first enantiomeric potency comparison based on ED_{50} doses was determined from rats trained to discriminate either 0.2 or 0.4 mg/kg of *S*(−)-nicotine (s.c.) from saline. In both groups of rats, *S*(−)-nicotine was about 10 times more potent than *R*(+)-nicotine [63]. Other studies also have reported *S*(−)-nicotine to be more potent than *R*(+)-nicotine, although thorough dose-response tests have not always been conducted nor ED_{50} calculations been performed (Table 2). Moreover, only animals trained to discriminate *S*(−)-nicotine from saline have evaluated the effects of *S*(−)- and *R*(+)-nicotine. In those studies, stimulus generalization tests indicated that *S*(−)-nicotine was, at least, ~10 times more potent than *R*(+)-nicotine as measured by *S*(−)-nicotine-like responding; to date, racemic nicotine has not been evaluated. As such, two areas for future drug discrimination studies can be proposed. First, even though *R*(+)-nicotine is about 10 times less potent than *S*(−)-nicotine, it is still a very potent drug that has not been employed as a training stimulus. Stereoselective drug effects are not solely a property of a drug, but are related both to the drug and the specific pharmacological (biological and/or behavioral) activity being examined; i.e. different methods/assays can afford dissimilar results. Therefore, *R*(+)-nicotine should be studied as a discriminative stimulus in animals and the results of stimulus generalization and antagonism tests compared to known results already obtained with *S*(−)-nicotine as training stimulus. Second, racemic nicotine also should be targeted for study as a training drug. (±)-Nicotine is a mixture of equal amounts of its two

Table 2 *S*(−)-Nicotine as a discriminative stimulus and comparative effects of nicotine stereoisomers

Species and *S*(−)-nicotine		Potency ratio	
Training dose	ED_{50} dose	S > R	Reference
Rat [0.2 mg/kg; (s.c.)]	(−)-Nicotine (0.083 mg/kg) (+)-Nicotine (0.764 mg/kg)	9.2	Meltzer et al. [63]
Rat [0.4 mg/kg; (s.c.)]	(−)-Nicotine (0.129 mg/kg) (+)-Nicotine (1.318 mg/kg)	10.2	Meltzer et al. [63]
Rat [0.4 mg/kg; (s.c.)]	(−)-Nicotine (0.11 mg/kg) (+)-Nicotine (ED_{50} not stated)	ND[a]	Romano et al. [64]
Squirrel monkey [0.032 or 0.065 mg/kg; (i.v.)]	(−)-Nicotine (0.015 mg/kg) (+)-Nicotine (0.44 mg/kg)	29	Takada et al. [44]
Rat [0.1 mg/kg; (s.c.)][b]	(−)-Nicotine (0.036 mg/kg) (−)-Nicotine (0.054 mg/kg) (+)-Nicotine (ED_{50} not stated)	10–20[c]	Goldberg et al. [65]
Rat [0.3 mg/kg; (i.p.)]	(−)-Nicotine (ED_{50} not stated) (+)-Nicotine (ED_{50} not stated)	3–10[c]	Brioni et al. [66]

[a]Isomer potency ratio not determined
[b]Two groups of rats were trained to discriminate 0.1 mg/kg of (−)-nicotine from saline
[c]Estimated potency ratio

enantiomers (Fig. 1) and comparative drug discrimination results of (±)-, *S*(−)- and *R* (+)-nicotine, when taken together, could (a) indicate a role for complex stereochemical effects of nicotine and (b) provide a unique perspective on mechanisms of action of nicotine.

5 Tobacco Alkaloids and Nicotine Metabolism

Chemical constituents in tobacco leaf exceed 4,000 and smoke from a burning cigarette contains over 7,000 substances from many chemical classes (e.g., [67–70]). The tobacco leaf contains many alkaloids and in fresh *Nicotina tabacum* (the leaf species most commonly used for the production of cigarette tobacco) the average alkaloid mixture typically consists of 93% *S*(−)-nicotine, 3.9% *S*(−)-anatabine, 2.4% *S*(−)-nornicotine, and 0.5% *S*(−)-anabasine (e.g., [4, 5, 71]; Fig. 2; but see [72]). In comparison, a typical *tobacco cigarette* contains approximately 1.5% of *S*(−)-nicotine, which constitutes ≥95% of alkaloid content [73]. In addition, some of the alkaloid content of tobacco leaf is decomposed during drying and fermentation, leading to substances such as myosmine, *S*(−)-cotinine and others (e.g., [74–77]). There is little doubt, however, that *S*(−)-nicotine is the major component responsible for the appeal of tobacco-based products.

In the body, nicotine is extensively metabolized and is susceptible to a significant first-pass effect during which 80–90% of it is metabolized by the liver. Also, the lung is able to metabolize nicotine, but to a much lesser degree [78, 79]. In humans, about 70–80% of nicotine is converted to the primary metabolite (−)-cotinine, a lactam derivative (Fig. 2). As mentioned earlier, (−)-cotinine also is a minor alkaloid found in tobacco leaf and is often used as a biomarker to detect tobacco use because of its relatively long half-life compared to that of (−)-nicotine. Another primary metabolite of nicotine is nicotine *N*′-oxide, although only about 4–7% of (−)-nicotine absorbed by smokers is metabolized to this product [80, 81]. Lastly, *S*(−)-nornicotine is a minor metabolite of nicotine and, as

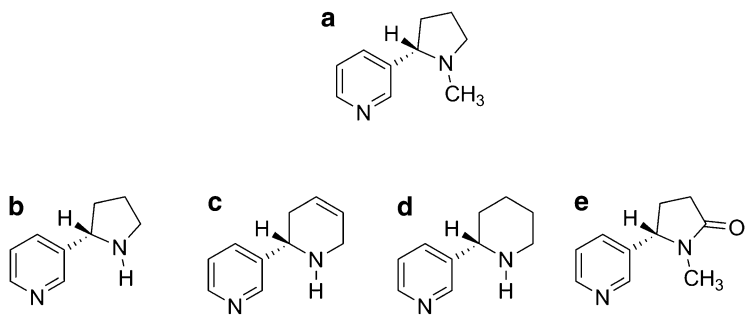

Fig. 2 Structural comparison of *S*(−)-nicotine (**a**) and related tobacco alkaloids *S*(−)-nornicotine (**b**), *S*(−)-anatabine (**c**), *S*(−)-anabasine (**d**), and *S*(−)-cotinine (**e**)

mentioned previously, is considered a minor alkaloid of tobacco (Fig. 2). Interestingly, however, in some varieties of tobacco, *S*(−)-nornicotine concentration exceeds that of *S*(−)-nicotine [82].

In drug discrimination studies, the activity and potency of metabolites have been shown to be important considerations in evaluations of the stimulus properties of drugs (for review, see [34]). Table 3 reviews the results of tobacco alkaloids and/or nicotine metabolites after their administration to animals trained to discriminate (−)-nicotine from saline. As can be seen, only one study has convincingly demonstrated (−)-

Table 3 *S*(−)-Nicotine as a discriminative stimulus: results of stimulus generalization and stimulus antagonism tests with racemic mixtures or stereoisomers of tobacco alkaloids and/or (−)-nicotine metabolites

Training dose of (−)-nicotine (route)	Species	Result[a]	Reference
S(−)-Cotinine			
0.2 mg/kg (s.c.)	Rat	PG (36%; s.c.)	Rosecrans et al. [83]
0.2 mg/kg (s.c.)	Rat	PG (47%; i.v.t.)[b]	Rosecrans et al. [83]
0.1 mg/kg (s.c.)	Rat	G[c]	Goldberg et al. [65]
0.032 or 0.065 mg/kg (i.v.)	Squirrel monkey	G	Takada et al. [44]
0.4 mg/kg (s.c.)	Rat	PG (74%; i.v.t.)	Rosecrans and Chance [48]
(±)-Nornicotine			
0.1 mg/kg (i.p.)	Rat	PG (76%)	Desai et al. [84]
0.32 mg/kg (i.p.)	Mouse	G	Caine et al. [85]
S(−)-Nornicotine			
0.1 mg/kg (s.c.)	Rat	G	Goldberg et al. [65]
0.032 or 0.065 mg/kg (i.v.)	Squirrel monkey	G	Takada et al. [44]
R(+)-Nornicotine			
0.1 mg/kg (s.c.)	Rat	G	Goldberg et al. [65]
(±)-Anabasine			
0.4 mg/kg; (s.c.)	Rat	G	Romano et al. [64]
0.032 or 0.065 mg/kg (i.v.)	Squirrel monkey	G	Takada et al. [44]
0.3 mg/kg (i.p.)	Rat	PG (~75%)	Brioni et al. [66]
0.32 mg/kg (i.p.)	Mouse	PG (~75%)	Caine et al. [85]
S(−)-Anabasine			
0.1 mg/kg (s.c.)	Rat	G	Stolerman et al. [55]
0.2 mg/kg (s.c.)	Rat	PG (~60%)	Stolerman et al. [55]
0.4 mg/kg (s.c.)	Rat	PG (~57%)	Stolerman et al. [55]
0.4 mg/kg (s.c.)	Rat	NA	Stolerman et al. [55]
0.4 mg/kg (s.c.)	Rat	PG (~60%)	Pratt et al. [50]
(±)Anatabine			
0.32 mg/kg (i.p.)	Mouse	G	Caine et al. [85]

[a]*PG* partial generalization, *G* stimulus generalization *NA* no stimulus antagonism
[b]*i.v.t.* intraventricular route of administration
[c]Cotinine sample was reported to be significantly contaminated with (−)-nicotine

nicotine stimulus generalization to (−)-cotinine and that occurred only at relatively high doses of drug [44]. Thus, *S*(−)-cotinine, the major metabolite of nicotine, does not appear to exert a significant role in the stimulus properties of (−)-nicotine. However, this conclusion does not rule out the possibility that (−)-cotinine may exert stimulus properties of its own that differ from those of (−)-nicotine and/or that occur at much lower doses of (−)-cotinine than doses that produced (−)-nicotine-like responding.

In comparison, *S*(−)-nicotine stimulus generalization occurred not only to "natural" *S*(−)-nornicotine, a minor metabolite of nicotine, but also to *R*(+)- and (±)-nornicotine (Table 3); however, these drugs were not as potent as (−)-nicotine [44, 65, 84, 85]. In the Goldberg et al. [65] study, *S*(−)- and *R*(+)-nornicotine produced dose response functions and ED_{50} values that were nearly identical, but unfortunately racemic nornicotine was not tested. Nevertheless, these data are of added interest. That is, nornicotine is a chiral substance and it would not be unusual to expect that one of its stereoisomers would (predominately) exhibit the targeted pharmacologic activity [i.e., (−)-nicotine-like responding] and that its antipode would be less potent, inactive, or exhibit a different type of biological/behavioral activity. In this study, however, the optical isomers of nornicotine did not exhibit any of the latter outcomes. Specifically, both isomers produced equally potent percent (−)-nicotine-like responding. This suggests that each isomer of nornicotine would contribute equally to (an expected) (−)-nicotine-like response that would be produced by (±)-nornicotine. Therefore, *S*(−)-, *R*(+)- and (±)-nornicotine should be evaluated in future DD studies of (−)-nicotine to explore what might be complex steric interactions in regard to their production of *S*(−)-nicotine-like responding.

Also of interest are the effects of anabasine and anatabine. (−)-Nicotine-trained animals exhibited very high partial or complete generalization to racemic anabasine but mostly partial generalization to "natural" *S*(−)-anabasine, except in animals trained to discriminate 0.1 mg/kg of (−)-nicotine from saline (Table 3). These results suggest that the untested *R*(+)-isomer of anabasine could produce, to some degree, marked (−)-nicotine-like effects; this possibility should be evaluated in future studies. Lastly, (−)-nicotine-trained mice generalized completely to (±)-anatabine but, unfortunately, "natural" *S*(−)-anatabine and its enantiomer were not tested (Table 3).

An important advisory from studies of the previously mentioned drugs is that results from racemic mixtures and optical isomers are not interchangeable. For example, the "natural" alkaloid substances in tobacco are reported to be stereoisomers that exhibit the *S*-configuration and levorotatory rotation. The three forms of drug [(±)-, *S*(−)- and *R*(+)-forms)] should be viewed as separate chemical entities and results obtained with one substance should not be used as substitute data for the (untested) other two drugs. For example, definitive conclusions about the activity/potency of an untested *S*(−)-enantiomer should not be drawn from results obtained from its racemic mixture or *R*(+)-isomer.

In summary, (−)-nicotine stimulus generalization tests of tobacco alkaloids and nicotine metabolites indicated that nicotine-like stimulus effects were produced by *S*(−)-cotinine, *S*(−)-nornicotine, and *S*(−)-anabasine, but these drugs were clearly

less potent than (−)-nicotine. The results strongly support the idea that (−)-nicotine is the most pharmacologically potent alkaloid in tobacco and that its stimulus effects are not due to the effects of a (more) potent metabolite. This does not, however, rule out the possibility that these other tobacco alkaloids and the metabolites of (−)-nicotine could exert some other kind of activity or that they may produce interactive effects in combination with (−)-nicotine.

6 Mechanism of Action

Historically, (−)-nicotine has facilitated our knowledge of the cholinergic nervous system (e.g., [13, 86–88]). It is now well established that nicotine binds to nicotinic acetylcholine receptors (nAChRs) at the cellular level and is the prototype drug used to classify nAChRs. These receptors belong to the super-family of ligand-gated ion channels that also includes $GABA_A$, $GABA_C$, glycine, and 5-HT_3 receptors [89–91]. In the mammalian brain, nAChRs are composed of α2–α7 and β2–β4 subunits with distribution patterns that appear to be distinct or to overlap (e.g., [92–94]). These subunits surround an ion channel and receptor binding by an agonist [e.g., (−)-nicotine] causes a closed (i.e., rest) conformation of the subunits to change to an open conformation, which allows inflow of sodium ions, and, consequently, produces cell depolarization (e.g., [95, 96]). (−)-Nicotine activates all brain nAChR subtypes, but binds preferentially and with high affinity to α4β2 nAChRs (e.g., [12]). Moreover, these subunits are thought to modulate the release of other neurotransmitters and this has led to the idea that nAChRs, at least in part, are located presynaptically (e.g., [97–100]). For example, (−)-nicotine may increase dopamine activity at some brain sites such as the nucleus accumbens, an area thought to be important to drugs of abuse (e.g., [14, 101, 102]; but see [16, 103]).

In drug discrimination studies, Schechter and Rosecrans [40] provided very strong, if not the strongest, evidence for the conclusion that the stimulus effects of (−)-nicotine were mediated centrally. In this study, rats trained to discriminate 0.4 mg/kg of (−)-nicotine from saline did not generalize (recognize) the administration of nicotine isomethonium iodide hydroiodide, a quaternary amine analog of nicotine that produces the peripheral, but not the central effects of nicotine because of poor penetration into the CNS. In addition, Schechter and Rosecrans [39] and Rosecrans et al. [104] reported on a series of studies in which cannulae were implanted into the dorsal hippocampus of control, dopamine (DA)-depleted or norepinephrine (NE)-depleted rats. These three groups of rats had previously been trained to discriminate 0.4 mg/kg of (−)-nicotine (s.c.) from saline and this discrimination was maintained fully in the control group but was somewhat lessened (but still maintained) in both the DA- and NE-depleted animals after peripheral administration of the training treatments (following surgery). However, when rats were injected with (−)-nicotine (1 μg) bilaterally into the hippocampus, the discrimination was markedly weakened in NE-depleted rats and was not observed in DA-depleted rats. Taken together, these results suggested that the reduced

"strength" of the stimuli in catecholamine-depleted animals provided support, at least in part, for the involvement of DA and NE in the stimulus properties of (−)-nicotine. Lastly, (−)-nicotine also may alter other neuronal systems that are related to substance use and abuse, such as opioid, glutamate, serotonin, and glucocorticoid (e.g., [97–99]).

Other drug discrimination studies of (−)-nicotine have been performed to determine its mechanisms of action. For example, stimulus antagonism tests of nicotine have been studied by three general approaches and the results of such studies are summarized in Tables 4 and 5. In one approach, doses of a receptor antagonist are combined with the training dose of nicotine to determine whether the stimulus effect can be blocked. If a drug is an effective antagonist of (−)-nicotine, then a dose related antagonism will occur in the animals' percentage of (−)-nicotine-appropriate responding (i.e., lever pressing does not stop, but occurs on the saline-designated lever). In a second technique, the dose response of (−)-nicotine is determined in both the presence and absence of a constant dose of the receptor antagonist. If the antagonism is competitive, then the dose response of nicotine will shift in a rightward and parallel manner. In a third tactic, various doses of (−)-nicotine are combined with various doses of a receptor antagonist. This method generates a series of nicotine/antagonist dose response curves and provides the most comprehensive picture of the interactions between the drugs. All three of these approaches have been employed to evaluate putative receptor antagonists of (−)-nicotine stimuli.

The results of antagonism tests typically fall into one of three categories: (a) complete antagonism (i.e., saline- or vehicle-like responding); (b) partial antagonism (i.e., 40 to ~70% drug-appropriate responding), and (c) no antagonism (i.e., $\geq$80% drug-appropriate responding). In tests that result in no stimulus antagonism, subjects respond $\geq$80% on the nicotine-designated lever after administration of doses of a receptor antagonist in combination with the training dose of nicotine. Such results indicate that percent (−)-nicotine-appropriate responding is still like that of the dose of training drug and that the receptor antagonist does not interfere with the neurochemical mechanisms that are important for the discrimination. In cases of partial stimulus antagonism, subjects respond "moderately" 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 stimulus effect(s) of the dose of training drug but also somewhat "saline-like." However, the saline-designated lever is also the default lever and subjects will press it under the saline (i.e., inert) condition or if the combination of drugs produces a stimulus effect(s) that is sufficiently dissimilar from that of the dose of training drug. Consequently, this type of data can be most difficult to interpret. Lastly, in cases of complete stimulus antagonism, subjects respond in a manner that is appropriate for the vehicle condition after administration of an appropriate dose of receptor antagonist in combination with the training dose of nicotine – but see discussion below of third state hypothesis.

Over the past 45 years, (−)-nicotine has been the subject of numerous attempts to block its stimulus effects and such tests have indicated quite clearly that nicotine

Table 4 *S*(−)-Nicotine as a discriminative stimulus: antagonism by mecamylamine

Training dose of (−)-nicotine (route)	Species	Result[a]	Reference
0.2 mg/kg (s.c.)	Rat	A	Morrison and Stephenson [35]
0.4 mg/kg (s.c.)	Rat	A	Schechter and Rosecrans [36, 37]
0.4 mg/kg (s.c.)	Rat	A	Schechter and Rosecrans [38]
0.2 mg/kg (s.c.)	Rat	A	Hirschhorn and Rosecrans [53]
0.4 mg/kg (s.c)	Rat	A	Hirschhorn and Rosecrans [53]
0.2 mg/kg (s.c.)	Rat	A	Chance et al. [105]
0.2 mg/kg (s.c.)	Rat	A	Meltzer et al. [63]
0.4 mg/kg (s.c.)	Rat	A	Meltzer et al. [63]
0.4 mg/kg (s.c.)	Rat	A	Romano et al. [64]
0.4 mg/kg (s.c.)	Rat	A	Stolerman et al. [106]
0.1 mg/kg (s.c.)	Rat	A	Stolerman et al. [55]
0.5 mg/kg (p.o.)	Rat	A[b]	Craft and Howard [107]
0.4 mg/kg (s.c.)	Rat	A	Stolerman et al. [108]
0.4 mg/kg (s.c.)[c]	Rat	A	Stolerman and Garcha [54]
0.5 mg/kg (s.c.)	Rat	A[d]	Miyata et al. [109]
0.5 mg/kg (s.c.)	Rat	A[d]	Ando et al. [110]
0.5 mg/kg (s.c.)	Rat	NA[e]	Ando et al. [110]
0.4 mg/kg (s.c.)	Rat	A	James et al. [28]
0.3 mg/kg (s.c.)	Rat	A	Brioni et al. [66]
0.2 mg/kg (s.c.)	Rat	A	Chandler and Stolerman [111]
1.6 mg/kg (s.c.)	Mouse	A	Stolerman et al. [43]
0.1 mg/kg (s.c.)	Rat	A	Gasior et al. [112]
0.4 mg/kg (s.c.)	Rat	A	Gasior et al. [112]
0.02 mg/kg (nasal spray)	Human	A	Perkins et al. [42]
0.4 mg/kg (s.c)	Rat	A	Mansbach et al. [113]
0.4 mg/kg (s.c.)	Rat	A	Wiley et al. [114]
0.6 mg/kg (s.c.)	Rat	A	Young and Glennon [115]
0.4 mg/kg (s.c.)	Rat	A	Zaniewska et al. [116]
0.4 mg/kg (i.p.)	Rat	A	Paterson et al. [117, 118]
0.32 mg/kg (s.c.)	Rat	A	Jutkiewicz et al. [119]
1.78 mg/kg (s.c.)	Rat	A	Jutkiewicz et al. [119]
1.78 mg/kg (s.c.)	Rhesus monkey	A	Cunningham et al. [120]
0.56 mg/kg (s.c.)	Mouse	A	Cunningham and McMahon [121]
1.0 mg/kg (s.c.)	Mouse	A	Cunningham and McMahon [121]
1.78 mg/kg (s.c.)	Mouse	A	Cunningham and McMahon [121]

[a]*A* stimulus antagonism, *NA* no stimulus antagonism
[b]Mecamylamine administered orally

(continued)

Table 4 (continued)

[c]Three groups of rats trained at 0.4 mg/kg s.c. of (−)-nicotine with different pre-session injection intervals
[d]Mecamylamine injected into nucleus accumbens blocked the systemic administration of (−)-nicotine
[e]Mecamylamine injected into ventral tegmental area or dorsal hippocampus did not block the systemic administration of (−)-nicotine

Table 5 *S*(−)-Nicotine as a discriminative stimulus: results of antagonism tests with dihydro-β-erythrodine (DHβE) and methyllycaconitine (MLA)

Training dose of (−)-nicotine (route)	Species	Result[a]	Reference
		DHβE	
0.1 mg/kg (s.c.)	Rat	A	Stolerman et al. [122]
0.4 mg/kg (s.c.)	Rat	A	Stolerman et al. [122]
0.4 mg/kg (s.c.)	Mouse	A	Gommans et al. [123]
0.2 mg/kg (s.c.)	Rat	A	Shoaib et al. [124]
0.4 mg/kg (s.c.)	Rat	A	Zaniewska et al. [116]
0.4 mg/kg (i.p.)	Rat	A	Paterson et al. [117, 118]
0.32 mg/kg (s.c.)	Rat	A	Jutkiewicz et al. [119]
1.78 mg/kg (s.c.)	Rat	A	Jutkiewicz et al. [119]
1.78 mg/kg (s.c.)	Rhesus monkey	NA	Cunningham et al. [120]
0.56 mg/kg (s.c.)	Mouse	A	Cunningham and McMahon [121]
1.0 mg/kg (s.c.)	Mouse	NA	Cunningham and McMahon [121]
1.78 mg/kg (s.c.)	Mouse	A	Cunningham and McMahon [121]
0.4 mg/kg (s.c)	Rat	A	Lee et al. [125]
		MLA	
0.3 mg/kg (i.p.)	Rat	NA	Brioni et al. [66]
0.4 mg/kg (s.c.)	Mouse	NA	Gommans et al. [123]
0.4 mg/kg (s.c.)	Rat	NA	Zaniewska et al. [116]
0.8 mg/kg (s.c.)	Mouse	PA (~50%)	Quarta et al. [126]
0.4 mg/kg (i.p.)	Rat	NA	Paterson et al. [117, 118]
0.4 mg/kg (s.c)	Rat	NA	Lee et al. [125]

[a]*A* stimulus antagonism, *NA* no stimulus antagonism, *PA* partial antagonism

exerts its stimulus effect, at least in part, through an interaction at nicotinic receptors in brain and, in particular, at a subtype of nicotinic receptor termed α4β2 receptors. This conclusion is based on the fact that the stimulus effects of nicotine are convincingly blocked by (a) mecamylamine, a voltage dependent noncompetitive channel blocker at nicotinic receptors (Fig. 3; Table 4) and (b) dihydro-β-erythrodine (DHβE), a nicotinic receptor antagonist that shows high affinity for the nAChR α4β2 subunit (Fig. 3; Table 5) but not by methyllycaconitine (MLA), a α7 nicotinic receptor antagonist (Table 5).

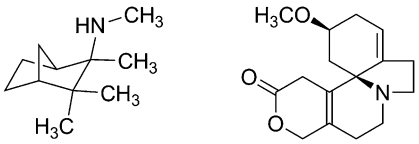

Fig. 3 Structures of nicotinic receptor antagonists mecamylamine (*left*) and dihydro-β-erythroidine (DHβE; *right*)

6.1 Mecamylamine

Mecamylamine (Inversine®, Vecamyl®; Fig. 3) was developed over 60 years ago and marketed as a ganglionic blocker for the treatment of hypertension (e.g., [127]). However, it is rarely used today for this purpose because of parasympathetic and sympathetic ganglia-related adverse effects (e.g., blurred vision, dry mouth, and dizziness). In addition, mecamylamine can produce CNS effects that include tremor, mental confusion, seizures, mania, and depression but the mechanisms by which these effects are produced are unclear. Also, mecamylamine is sometimes used as an anti-addictive drug to help people stop smoking tobacco products (e.g., [128, 129]). However, an early study of mecamylamine in human smokers reported an increased rate (30%) of smoking, which was regarded as evidence for self-titration of (−)-nicotine [130].

Biochemical and pharmacological studies have characterized mecamylamine as a nonselective, voltage dependent and noncompetitive receptor antagonist of neuronal nAChRs and it is often referred to as a "nicotine receptor antagonist." As such, mecamylamine probably exerts its effects via interaction with sites distinct from nAChR agonist binding sites and, therefore, does not compete with (−)-nicotine for binding. For example, some biochemical studies suggest that mecamylamine is a channel blocker that inhibits most neuronal nAChRs (e.g., [131–133]). Table 4 shows that mecamylamine antagonism of (−)-nicotine discriminative stimuli has been consistently demonstrated in many studies. In general, these studies employed mecamylamine at 1–3 mg/kg to block the stimulus effects of nicotine (0.1–1.78 mg/kg). In addition, some of these studies have confirmed the non-competitive antagonism character of mecamylamine because the antagonism effects were not always reversed or surmounted by higher doses of (−)-nicotine (e.g., [106]).

6.2 DHβE (Dihydro-β-Erythroidine)

DHβE (Fig. 3) is an alkaloid found in plant seeds of *Erythrina* and is a competitive nAChR receptor antagonist with a preference for neuronal β2 subtypes. For example, DHβE (at nM concentrations) blocks α4β2 and α3β2 nAChRs but is much less potent at α3β4 and α7 nAChRs expressed in Xenopus oocytes (e.g., [134–137]). DHβE interacts reversibly with nAChRs at, or close to, the agonist binding site(s),

stabilizes the receptor in a conformation with the channel closed and prevents access for receptor agonists; however, this blockade is surmountable with increased agonist [e.g., (−)-nicotine] concentrations (e.g., [123]). DHβE has been employed in stimulus antagonism studies of (−)-nicotine to assess involvement of the α4β2 nAChR subunit. Research results summarized in Table 5 indicate that DHβE effectively blocked the stimulus effects of (−)-nicotine in rats or mice (but see exceptions reported by [120, 121]). In addition, and in contrast to mecamylamine, DHβE antagonism of the stimulus effect of (−)-nicotine was reversed (competitively) by increased doses of (−)-nicotine (e.g., [122]).

6.3 Methyllycaconitine (MLA)

Methyllycaconitine (MLA) is an alkaloid found in many plant species of *Delphinium* (larkspurs) and is generally toxic to animals (e.g., [138]). Its biochemical pharmacology indicates that it is a relatively potent competitive receptor antagonist that is selective for α7 nAChRs (e.g., [139–141]). MLA has been used in stimulus antagonism studies of (−)-nicotine to assess potential involvement of α7 nAChRs. Table 5 presents results of MLA/(−)-nicotine combination studies and shows that MLA failed to alter the stimulus effects of (−)-nicotine in rats or mice (but see partial antagonism reported by Quarta et al. [126]).

6.3.1 Summary and Analysis of Antagonism Results

Mecamylamine and DHβE have repeatedly been shown to produce complete antagonism of the stimulus effects of (−)-nicotine. However, the resultant responding on the saline-designated lever may, but does not necessarily, indicate that the combination of substances produced an inert effect. The possibility exists that the effects of either (or both) receptor antagonist in combination with the training dose(s) of nicotine are not like those of the training dose of nicotine nor like the vehicle (i.e., inert) condition. In such cases, the saline-designated lever would have served as the "default" response [a.k.a. "transfer test over-inclusiveness" (see [142]) or 'third-state hypothesis" (see [143])]. Thus, results of antagonism tests only indicated that the stimulus effects produced by the combination of antagonists and training doses of (−)-nicotine are dissimilar from those produced by the training dose of (−)-nicotine (alone). In this regard, there is some drug discrimination data that suggest the need for tests to determine if a "third state hypothesis" explanation could account for results when mecamylamine (or DHβE) is combined with (−)-nicotine. For example, Garcha and Stolerman [144] trained rats to discriminate mecamylamine (3.5 mg/kg s.c.) from saline. The mecamylamine stimulus generalized completely to the ganglion blockers pentolinium and pempidine but not to hexamethonium, trimetaphan, or chlorisondamine. Mecamylamine stimulus generalization also did not occur to (−)-nicotine, atropine, or

scopolamine. In antagonism tests, (−)-nicotine failed to block the stimulus effects of mecamylamine. In another study, Cunningham et al. [145] trained rhesus monkeys to discriminate 5.6 mg/kg of mecamylamine from saline and reported that the mecamylamine stimulus was not blocked by (−)-nicotine nor did (−)-nicotine produce mecamylamine-like responding. Mecamylamine stimulus generalization did occur, however, to the peripherally mediated nicotinic receptor antagonist hexamethonium (at a relatively high dose), a quaternary drug that does not readily penetrate into the CNS. It should be noted that hexamethonium, at relatively low doses, does not block the stimulus effects of (−)-nicotine but when administered at high doses has occasionally been reported to attenuate nicotine-like responding; probably the result of penetration into the CNS of a small proportion of the administered dose of drug (e.g., [35, 38, 64, 106, 146]).

Taken together with previous studies (Table 4), results indicate that (a) mecamylamine, by itself, can exert discriminative stimulus effects, (b) cross stimulus generalization does not occur between mecamylamine and (−)-nicotine regardless of which drug was used as training stimulus and (c) asymmetric antagonism occurred between (−)-nicotine and mecamylamine; i.e. mecamylamine blocked the discriminative stimulus effects of nicotine but not vice versa. The latter results could be accounted for by the possibility of dose-dependent effects of mecamylamine such that different doses produced qualitatively or mechanistically different pharmacological effects. That is, relatively low doses (i.e., 1–3 mg/kg) of mecamylamine are used routinely in studies to block the stimulus effects of (−)-nicotine. However, both Garcha and Stolerman [144] and Cunningham et al. [145] reported that low training doses (1–3 mg/kg) of mecamylamine were not sufficient to produce stable discrimination learning and, thus, higher doses (3.5 mg/kg and 5.6 mg/kg, respectively) of drug had to be employed. This suggests that different doses of mecamylamine might produce, to some degree, dissimilar pharmacological (i.e., stimulus) effects. Follow-up studies should evaluate the argument that the dose of mecamylamine can determine its "distinctiveness" in drug discrimination studies. Other studies should be designed to test the third state hypothesis to ascertain if subjects can be trained to discriminate a *drug mixture* of a (reported) dose of mecamylamine that blocked a (reported) dose of (−)-nicotine from saline vehicle. If animals cannot learn to discriminate the drug mixture from saline, then this would be evidence that the drug combination likely exerts an inert stimulus effect in the animals; i.e. negative data that would argue against a third state effect to explain mecamylamine antagonism of (−)-nicotine. On the other hand, if animals can discriminate the drug mixture from saline, then this suggests that the combination of drugs produces a stimulus effect that is unlike (−)-nicotine and, consequently, results in responding on the saline-designated (default) lever in (−)-nicotine-trained animals. Alternatively, or additionally, a three-choice operant conditioning procedure could be employed to examine the stimulus effects of (−)-nicotine versus mecamylamine versus saline vehicle to assess qualitative and/or quantitative similarities/differences in actions. Thus, if a group of subjects can be trained to discriminate a dose of mecamylamine versus a dose of (−)-nicotine versus saline, then this could lead to a more precise characterization of

the stimulus properties of the treatment conditions. The interested reader is referred to Frey and Winter [143] and Glennon and Young [34] for further discussion of third state hypothesis.

7 Pharmacotherapies for Smoking Cessation

> Giving up smoking is the easiest thing in the world. I know because I've done it thousands of times.
> – Mark Twain

When a nicotine-dependent user tries to quit nicotine consumption, cessation of use is typically followed by a withdrawal period that may last for months and includes symptoms that can lead an individual to relapse. (−)-Nicotine withdrawal symptoms might begin within a few hours after the last nicotine product, and include irritability/anger/stress/anxiety, sleep disturbances, depressed mood, craving, cognitive and attention deficits, and increased appetite. Symptoms may last a few days or persist for months or longer. Unfortunately, most smokers relapse within just a few days, and less than 10% of those who try to quit on their own achieve more than a year of abstinence; thus, quitting the nicotine product often requires multiple attempts (e.g., [147]).

One of the most common smoking-cessation treatments is nicotine-replacement therapy (NRT), when smokers simply substitute (−)-nicotine inhaled via cigarettes with, ironically, "safer formulations" of (−)-nicotine. In fact, nicotine itself was the first pharmacological agent approved by the U.S. Food and Drug Administration (FDA) for use in smoking cessation therapy. NRT formulations include gum, patch, inhaler, spray, lozenge, and e-cigarette. These products can maintain reinforcement effects of (−)-nicotine at (gradually) reduced doses and concomitantly reduce withdrawal symptoms associated with cessation. The goal is to gradually diminish the body's ingestion of nicotine, establish remission, and sustain it long enough for the ex-smoker to develop "coping" strategies to avoid relapse (for review, see [148]). These nicotine delivery systems are thought to be equally effective, with about 20% of those that received therapy not smoking at 1 year and up to 10% remaining non-smokers if treatment is continued [148, 149]. Other products that have been (are) promoted for cessation of (−)-nicotine consumption include (−)-lobeline (unapproved by the FDA) and the FDA approved products bupropion (Zyban®) and varenicline (Chantix®).

7.1 (−)Lobeline

(−)Lobeline (Fig. 4) is a natural substance found in, for example, "Indian tobacco" (*Lobelia inflata*) and "Devil's tobacco" (*Lobelia tupa*). Lobeline binds with high affinity to α4β2 nAChRs and displays mixed receptor agonist/antagonist actions

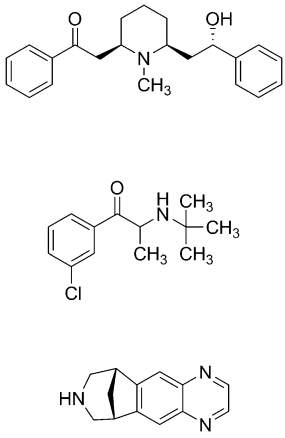

Fig. 4 Structures of pharmacotherapies that have been [(−)-lobeline (*top*)] or are promoted [bupropion (*middle*) and varenicline (*bottom*)] for cessation of (−)-nicotine consumption

(e.g., [150–154]). At one time, lobeline was promoted (but not FDA approved) for smoking cessation in several products: CigArrest®, Bantron®, and Nicoban®. However, the FDA removed these products from the US marketplace in 1993 until such time that they could be shown to be efficacious in scientific studies of smoking cessation (see [155–157]). In drug discrimination studies, (−)-lobeline has been utilized only as a test drug. For example, in rats and squirrel monkeys trained to discriminate cocaine, *S*(+)-methamphetamine or (−)-nicotine from vehicle, stimulus generalization to lobeline occurred only in relatively "low dose" cocaine-trained rats at a relatively short pre-session injection interval [158]. Importantly, however, (−)-nicotine-trained rats have consistently shown that (−)-lobeline does not produce marked (−)-nicotine-like responding, which supports the argument of unproven efficacy of lobeline as a substitute for nicotine-like effects in smoking cessation treatment (Table 6). Lobeline has, however, been shown to antagonize partially the stimulus effects of (−)-nicotine and *S*(+)-methamphetamine ([64, 160]; but see [66]). Surprisingly, and unfortunately, there does not appear to be any reports in the literature of (−)-lobeline as a training stimulus in drug discrimination studies. Such studies could prove informative and might reveal important similarities and differences in stimulus effects between (−)-lobeline, (−)-nicotine, cocaine and *S*(+)-methamphetamine.

7.2 Bupropion

Bupropion [a.k.a. amfebutamone, (*RS*)-2-(*tert*-Butylamino)-1-(3-chlorophenyl)propan-1-one, 3-Chloro *tert*-butylcathinone, 3-Chloro-*N*-*tert*-butyl-β-ketoamphetamine; Fig. 4] is a phenylaminoketone or cathinone derivative that is a weak central nervous system (CNS) stimulant. It is prescribed as medication for the treatment of depression (Wellbutrin®) and/or as an adjunct in smoking cessation therapy (Zyban®). In fact, the application of bupropion for smoking cessation was first noted serendipitously by

Table 6 Effects of (−)-lobeline as a test drug in drug discrimination studies

Training drug	Species	Result[a]	Reference
Cocaine[b]	Rat	G	Cunningham et al. [158]
Cocaine[c]	Rat	NG	Desai et al. [159]
S(+)Methamphetamine[d]	Rat	NG, PA	Miller et al. [160]
S(+)Methamphetamine	Squirrel monkey	NG (~30%)	Desai and Bergman [161]
(−)-Nicotine	Rat	NG	Schechter and Rosecrans [40]
(−)-Nicotine	Rat	NG (34%; s.c.)	Rosecrans et al. [83]
(−)-Nicotine	Rat	PG (48%; i.v.t.)	Rosecrans et al. [83]
(−)-Nicotine[e]	Rat	NG, PA	Romano et al. [64]
(−)-Nicotine	Rat	NG (~36%)	Reavill et al. [162]
(−)-Nicotine	Rat	NG, NA	Brioni et al. [66]

[a]*G* stimulus generalization, *NG* no stimulus generalization, *PA* partial stimulus antagonism, *NA* no stimulus antagonism

[b]Separate groups of rats trained to discriminate either 1.6 or 5 mg/kg of cocaine from saline. Both cocaine training stimuli generalized to lobeline but only with a relatively short (10 min) pre-session injection interval

[c]No generalization in rats trained to discriminate 8.9 mg/kg of cocaine from saline

[d](−)-Lobeline reduced %*S*(+)-Methamphetamine-appropriate responding from 100% to approximately 60%

[e]T-maze study. (−)-Nicotine stimulus did not generalize to lobeline, but lobeline did produce partial antagonism of the (−)-nicotine stimulus

clinical observations that depressed patients who received the drug decreased their smoking of tobacco (e.g., [163]). Follow-up studies showed that the administration of bupropion, in combination with counseling, produced comparable efficacy to NRTs at the 1-year benchmark for smoking cessation (e.g., [149, 164–166]). Its mechanisms of action (for both indications), however, are not known with certainty but bupropion may produce indirect agonist effects, at least in part, via relatively weak norepinephrine/dopamine reuptake inhibition (NDRI) (e.g., [167–170]). Other studies have reported that bupropion blocked the acute effects of (−)-nicotine in a number of behavioral assays in mice (e.g., [171, 172]). In drug discrimination studies, bupropion has received limited attention as a training agent, but much attention as a test drug (Tables 7, 8, and 9).

To date, only three drug discrimination studies have employed bupropion as training drug and each of these studies used rats as subjects. Jones et al. [173] reported the first drug discrimination study of bupropion and demonstrated that 20 mg/kg, but not 5 or 10 mg/kg, of bupropion was effective as training dose. Two other studies used 17 or 40 mg/kg of bupropion as dose of training drug [174, 175]. Table 7 summarizes the results of these studies and indicates quite clearly that bupropion stimulus generalization occurred to other CNS stimulants and several catecholamine reuptake inhibitors. These drugs included (±)- and *S*(+)-amphetamine, caffeine, cocaine, methylphenidate, mazindol, SKF 82958, nomifensine, WIN 35428 and GBR 12909 (vanoxerine). In addition, bupropion stimuli produced (high) partial generalization to a number of direct or indirect

Table 7 Bupropion as a discriminative stimulus: drugs that produced complete bupropion-like stimulus effects (i.e., ≥80% bupropion-appropriate responding)[a]

Mechanism of action	Drug class or test drug
(±)-Amphetamine (Benzadrine®)	Stimulant
S(+)-Amphetamine (Adderall®)	Stimulant
Benocyclidine (BTCP)	Stimulant
	Dopamine Reuptake Inhibitor
Benzylpiperazine	Designer Drug (Stimulant)
Caffeine	Stimulant
Cocaine	Stimulant
EXP-561[b]	Norepinephrine/Dopamine/Serotonin
	Reuptake Inhibitor
GBR 12909 (Vanoxerine)	Dopamine Reuptake Inhibitor
GBR 12935[c]	Dopamine Reuptake Inhibitor
LU 17-133[d]	Norepinephrine/Dopamine Uptake Inhibitor
Mazindol (Mazanor®, Sanorex®)	Stimulant
	Norepinephrine/Dopamine/Serotonin
	Reuptake Inhibitor
Methylphenidate (Ritalin®)	Stimulant
Nomifensine (Merital®, Alival®)	Antidepressant
	Norepinephrine/Dopamine Reuptake Inhibitor
RU 24213[e]	Dopamine D_2 Receptor Agonist
SKF 82958[f]	Stimulant
	Dopamine D_1/D_5 Receptor Agonist
Viloxazine (Vivalan®, Vivarint®, Vicilan®)[g]	Antidepressant/Stimulant
	Norepinephrine Reuptake Inhibitor
WIN 35428 (β-CFT)[h]	Stimulant
	Dopamine Reuptake Inhibitor

[a]Data from rats trained to discriminate 20 mg/kg of bupropion [173], 40 mg/kg of bupropion [174] or 17 mg/kg of bupropion [175] from saline vehicle
[b]4-Phenylbicyclo[2.2.2]octan-1-amine
[c]1-(2-(Diphenylmethoxy)ethyl)-4-(3-phenylpropyl)piperazine
[d](±)-Trans-4-[3-(3,4-dichlorophenyl)-indan-1-yl]-1-piperazineethanol
[e]*N*-*n*-propyl-*N*-phenylethyl-4(3-hydroxyphenyl)ethylamine
[f]3-Allyl-6-chloro-1-phenyl-1,2,4,5-tetrahydro-3-benzazepine-7,8-diol
[g]Jones et al. [173] reported complete bupropion generalization to viloxazine but Blitzer and Becker [174] reported bupropion (high) partial generalization to viloxazine
[h](−)-2-β-Carbomethoxy-3-β-(4-fluorophenyl)tropane

receptor agonists of catecholamine systems such as indatraline, pergolide, SKF 38393, and viloxazine (Table 8). Table 8 also indicates that bupropion stimulus generalization did not occur to (a) antidepressants from other chemical classes such as amitriptyline, mianserin, desipramine or zimelidine, or (b) substances from other drug classes such as the anxiolytic diazepam (but see partial generalization to chlordiazepoxide in [173]), the hallucinogen LSD, the analgesic morphine, or the sedative pentobarbital [173–175]. Curiously, however, the stimulus effects of

Table 8 Bupropion as a discriminative stimulus: drugs that produced partial or no bupropion-like responding[a]

Test drug	Highest % of bupropion-appropriate responding	Drug class or mechanism of action
Amitriptyline (Elavil®)	~30	Antidepressant
Benztropine (Cogentin®)	41	Anti-Parkinson
Bromocriptine (Parlodel®, Cycloset®)	~20	Monoamine receptor agent
Chlordiazepoxide (Librium®)	~60	Antianxiety
Clonidine (Catapres®)	~20	α_2-Adrenergic/imidazoline
		Receptor agonist
Desipramine (Norpramin®, Pertofrane®)	~35	Antidepressant
Diazepam (Valium®)	~20	Antianxiety
Imipramine (Tofranil®)	~50	Antidepressant
Indatraline (LU 19-005)	71	Non-selective monoamine
		Transporter inhibitor
Isoproterenol (Isuprel®)	~15	Non-selective β-adrenergic
		Receptor agonist
LSD	~20	Hallucinogen
Mianserin (Tolvon®, Norval®)	~35	Antidepressant/anxianxiety
Morphine (MScontin®, Oramorph®)	~15	Analgesic
Nisoxetine	~20	Norepinephrine reuptake inhibitor
Nortriptyline (Aventyl®, Sensova®)	~60	Antidepressant
(−)-NPA[b]	~63	Dopamine D_2 agonist
Pentobarbital (Nembutal®)	0	Sedative/hypnotic
Pergolide (Permax®, Prascend®)	69	Non-selective dopamine/serotonin agonist
Phenethylamine	~50	Monoamine neuromodulator
(−)Quinpirole	57	Dopamine D_2/D_3 receptor agonist
Quipazine	~35	Non-selective serotonin receptor agent
Scopolamine (Transdermscop®)	~40	Anti-motion sickness
		Muscarinic receptor antagonist
SCH 23390[c]	<5	Dopamine D_1 receptor antagonist
SKF 38393[d]	67	Dopamine D_1/D_5 receptor partial agonist
SKF 75670[e]	52	Dopamine D_1 agent
SKF 77434[f]	65	Dopamine D_1 receptor partial agonist
Spiperone (Spiropitan®)	~40	Antipsychotic
Thyrotropin-Releasing Hormone (TRH)	~20	Hypothalamic hormone
Viloxazine (Vivalan®, Vivarint®)[g]	~70	Antidepressant/stimulant
		Norepinephrine reuptake inhibitor

(continued)

Table 8 (continued)

Test drug	Highest % of bupropion-appropriate responding	Drug class or mechanism of action
Zimelidine	<5	Antidepressant
		Selective serotonin reuptake inhibitor

[a]Results from Jones et al. [173], Blitzer and Becker [174] and Terry and Katz [175]
[b]*R*(−)-10,11-dihydroxy-*N*-*n*-propylnorapomorphine
[c]7-Chloro-3-methyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepin-8-ol
[d]1-Phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine-7,8-diol
[e]7,8-Dihydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine
[f]3-Allyl-1-phenyl-1,2,4,5-tetrahydro-3-benzazepine-7,8-diol
[g]Jones et al. [173] reported complete bupropion generalization to viloxazine but Blitzer and Becker [174] reported bupropion (high) partial generalization to viloxazine

bupropion were not blocked by monoamine receptor antagonists such as haloperidol, thioridazine, thiothixene, propranolol, phenoxybenzamine, and cyproheptadine but were blocked partially by spiperone (which also generalized partially) and completely by SCH 23390 [174, 175]. The general lack of antagonism of the stimulus effects of bupropion, especially by catecholamine receptor antagonists, was rather unexpected and suggests that additional (stimulus antagonism and/or generalization) studies be undertaken to more fully elucidate its mechanism of action.

In comparison, bupropion has received extensive evaluation as a test drug in studies that used animals trained to discriminate *S*(+)-amphetamine, clenbuterol, cocaine, ethanol, GBR 12909, imipramine, isoproterenol, MDMA ("Ecstasy"), *S*(+)-methamphetamine, methylphenidate, mirtazapine, (−)-nicotine, oxazepam, rimonabant or Δ^9-THC from vehicle (Table 9). The results of these studies showed clearly that *S*(+)-amphetamine-, cocaine-, or GBR 12909-trained animals generalized to bupropion, which indicates cross-generalization between bupropion and these drugs regardless of which drug was used as training stimulus (e.g., [173–176, 188]). However, in one study, Rush et al. [179] trained humans to discriminate *S*(+)-amphetamine from vehicle and reported only partial generalization to bupropion, but the lack of complete stimulus generalization may be related to administered doses of bupropion. That is, the dose range for the antidepressant effect of bupropion in humans is considered to be 300–750 mg and it is within this dose range where concerns have been raised about the occurrence of seizures and the possibility of drug-induced psychotic symptoms, the latter a noted risk when prescribing CNS stimulants (e.g., [168, 169, 203]). On the other hand, the anti-smoking doses of bupropion are stated to be 150 and 300 mg; doses above 300 mg are not recommended and, in fact, are discouraged [204]. However, doses up to 300–400 mg of bupropion usually do not exert marked CNS stimulant effects in humans. Miller and Griffith [205], for example, reported that the effects of bupropion up to 400 mg produced little resemblance to *S*(+)-amphetamine. They speculated that because bupropion is such a

Table 9 Results of bupropion as a test drug in animals trained to discriminate various training drugs from saline vehicle

Training drug	Species	Result[a]	Reference
S(+)-Amphetamine	Pigeon	G	Evans and Johanson [176]
S(+)-Amphetamine	Rhesus monkey	G	de la Garza and Johanson [177]
S(+)-Amphetamine	Rhesus monkey	G	Kamien and Woolverton [178]
S(+)-Amphetamine	Human	PG (40%)	Rush et al. [179]
S(+)-Amphetamine	Rat	G	Bondarev et al. [180]
S(+)-Amphetamine	Rat	G	Heal et al. [181]
S(+)-Methamphetamine	Rhesus monkey	G	Banks et al. [182]
Clenbuterol	Rat	NG	Makhay and O'Donnell [183]
Cocaine	Rat	G	Lamb and Griffiths [184]
Cocaine	Rhesus monkey	G	Kleven et al. [185]
Cocaine	Pigeon	G	Johanson and Barrett [186]
Cocaine	Rat	G	Broadbent et al. [187]
Cocaine	Rat	G	Baker et al. [188]
Cocaine	Rat	G	Quinton et al. [189]
Cocaine	Rat	G	Paterson et al. [117, 118]
Cocaine	Rat	G	Awasaki et al. [190]
Ethanol	Rat (P)[b]	PG (50%)	McMillan et al. [191]
Ethanol	Rat (NP)[b]	G	McMillan et al. [191]
GBR 12909 (Vanoxerine)	Squirrel monkey	G	Melia and Spealman [192]
Imipramine	Pigeon	G	Zhang and Barrett [193]
Isoproterenol	Rat	G	Crissman and O'Donnell [194]
MDMA ("Ecstasy")	Rat	NG	Mori et al. [195]
S(+)-Methamphetamine	Pigeon	G	Sasaki et al. [196]
S(+)-Methamphetamine	Rat	G	Munzar and Goldberg [197]
Methylphenidate	Rat	G	Mori et al. [195]
Mirtazapine	Rat	PG (40%)	Dekeyne and Millan [198]
(−)-Nicotine	Rat	G	Young and Glennon [115]
(−)-Nicotine	Rat	G	Wiley et al. [114]
(−)-Nicotine	Rat	PG (70%)	Desai et al. [159]
(−)-Nicotine	Rat	NG	Shoaib et al. [199]
(−)-Nicotine	Mouse	PG (70%)	Damaj et al. [200]
(−)-Nicotine	Rhesus monkey	NG (23%)	Cunningham et al. [120]
Oxazepam	Pigeon	NG	de la Garza et al. [201]
Rimonabant	Rhesus monkey	PG (45%)	Schulze et al. [202]
Δ^9-THC	Rhesus monkey	NG	Schulze et al. [202]

[a]*G* stimulus generalization, *PG* partial generalization, *NG* no stimulus generalization
[b]*P* ethanol preferring rats, *NP* non-ethanol preferring rats

weakly potent CNS stimulant they were possibly examining only the "lower portion" of the dose response curve for bupropion; however, if the dose(s) was elevated, then *S*(+)-amphetamine-like effects would have occurred. In the Rush et al. [179], only "lower" doses of 50–400 mg of bupropion were examined in human subjects trained

to discriminate the effect of 20 mg of *S*(+)-amphetamine from placebo. The administration of these doses of bupropion in substitution tests produced low to moderate levels of (+)-amphetamine-like responding, accompanied by subject-rated drug effects such as "alert/energetic," "elated," and "vigorous" that somewhat overlapped with those of *S*(+)-amphetamine. As such, complete *S*(+)-amphetamine stimulus generalization to bupropion may have occurred if relatively higher doses of bupropion had been administered (for further discussion, see [115]).

Table 9 also reveals that animals trained to discriminate (−)-nicotine from saline produced (high) partial generalization or complete generalization to bupropion; however, two studies reported no (−)-nicotine stimulus generalization to bupropion. Although future studies will be needed to explain the apparent discrepancy in results, evidence does suggest that (−)-nicotine and bupropion can share, to some degree, a similar stimulus effect. Consequently, bupropion may help some people refrain from smoking because it produces effects that serve as a suitable substitute for (−)-nicotine in the individual who is motivated to quit smoking. Lastly, the issue of bupropion as pharmacotherapy for cessation of nicotine consumption may be complicated by the actions of bupropion metabolites (e.g., [180, 200]). For example, chemical pathways of bupropion metabolism include hydroxylation of its *tertiary*-butyl group (with or without subsequent cyclization) and/or reduction of its carbonyl group to an alcohol (e.g., [206]). Moreover, species differences are known in the metabolism of bupropion (e.g., [207]). In humans, two major metabolites are a phenylmorpholinol, hydroxybupropion (BW 306U), and an aminoalcohol, *threo*hydrobupropion [sometimes referred to as *threo*dihydrobupropion, also known as *R*,*R*-2-(*tert*-butylamino)-1-(3-chlorophenyl)propanol or BW A494U] [206, 208–210]. Another human metabolite, although formed in lesser amounts than the others, is *erythro*hydrobupropion. The stimulus effects of bupropion metabolites (isomers) were examined in (−)-nicotine-trained rats and (+)- and (−)-threohydrobupropion substituted partially [180]. In contrast, *R*,*R*-hydroxybupropion produced vehicle-like responding in (−)-nicotine animals but, when given in combination with the training dose of (−)-nicotine, resulted in an attenuated nicotine-like effect. On the other hand, *S*,*S*-hydroxybupropion partially (66%) substituted for (−)-nicotine [180]. In another study, Damaj et al. [200] reported similar results. That is, a (−)-nicotine stimulus in mice partially generalized to *S*,*S*-hydroxybupropion but did not generalize to *R*,*R*-hydroxybupropion. Taken together, these results appear to indicate that some bupropion metabolites probably play a role(s) in the complex actions of this drug. Moreover, data also suggested that it is unlikely that any one metabolite (or isomer) is chiefly responsible for the stimulus actions of bupropion. In this regard, there does not appear to be any reports in the literature of bupropion metabolites (or isomers) being tested in bupropion-trained animals. Such data might give the best indication of the role each metabolite exerts in the stimulus properties of bupropion. In addition, bupropion stimulus generalization tests should be conducted with other pharmacotherapies used for smoking cessation, such as (−)-lobeline or varenicline, to assess potential similarities and/or differences in effects between these drugs.

7.3 *Varenicline*

Varenicline (Chantix®; Fig. 4) is prescribed as an adjunct medication in smoking cessation therapy and is thought to exert its effects as a partial agonist at α4β2 nAChRs and as a full agonist at α7 nAChRs [211, 212]. In drug discrimination studies, (−)-nicotine-trained rodents (rat or mouse) or non-human primates (rhesus monkeys) displayed a high degree of partial generalization or complete substitution to varenicline (Table 10). These data indicate that (−)-nicotine and varenicline share a similar stimulus effect. Thus, varenicline may assist people to refrain from smoking because it produces effects that serve as a suitable substitute for nicotine in the individual who wants to quit smoking. In comparison, *S*(+)-methamphetamine-trained animals generalized partially to varenicline, whereas cocaine-trained monkeys did not. Lastly, a search of the literature did not reveal any reports of varenicline as a training stimulus in drug discrimination studies. Such studies could provide important insights into the characterization of stimulus effects of varenicline in comparison to the degree of varenicline-like responses that might be produced by (−)-nicotine, cocaine, and/or *S*(+)-methamphetamine.

8 Summary and Conclusions

S(−)-nicotine is the pivotal reason that individuals persist in their consumption of smoke and smokeless nicotine-based products. The psychoactive effects of (−)-nicotine have been characterized as both "stimulant" and "calming." These effects are probably influenced by the mental status and expectations of the user. Thus,

Table 10 Varenicline as a test drug in animals trained to discriminate cocaine, S(+)-amphetamine or (−)-nicotine from saline

Training drug	Species	Result[a]	Reference
Cocaine	Rhesus monkey	NG	Gould et al. [213]
S(+)Methamphetamine	Rat	PG (~60%)	Desai and Bergman [161]
S(+)Methamphetamine	Squirrel monkey	PG (~50–65%)	Desai and Bergman [161]
S(+)Methamphetamine	Rhesus monkey	PG (~35–40%)	Banks et al. [182]
(−)Nicotine	Rat	PG (60%)	Smith et al. [214]
(−)Nicotine	Rat	G	Rollema et al. [215]
(−)Nicotine	Rat	PG (63%)	LeSage et al. [216]
(−)Nicotine	Rat	G	Paterson et al. [117, 118]
(−)Nicotine	Rat	G	Jutkiewicz et al. [119]
(−)Nicotine	Rhesus monkey	G	Cunningham et al. [120]
(−)Nicotine	Rat	G	Le Foll et al. [217]
(−)Nicotine	Mouse	PG (~50–70%)	Cunningham and McMahon [121]
(−)Nicotine	Mouse	PG (71%)	Rodriguez et al. [218]

[a]*G* stimulus generalization, *PG* partial generalization, *NG* no stimulus generalization

consumers of nicotine may experience alertness or relaxation and these effects could form the basis of the claim that "(-)-nicotine is a drug for all seasons." (−)-Nicotine produces effects that appear to be mediated primarily through α4β2 nicotinic acetylcholine receptors (nAChRs) with subsequent influence on other neurotransmitters systems (e.g., dopamine). This chapter has described the basic methodology and usefulness of drug discrimination procedures to study the effects of (−)-nicotine. In this assay, subjects are tasked to identify the effects of (−)-nicotine versus saline vehicle. (−)-Nicotine can serve as a discriminative stimulus in non-human animal and human subjects. The model exhibits stability, sensitivity and displays several advantages over acute behavioral techniques to study in vivo pharmacological effects of this drug. Once established, the (−)-nicotine stimulus can be demonstrated to be dose related, time dependent and stereoselective: *S*(−)-nicotine is more potent than *R*(+)-nicotine in the production of (−)-nicotine-appropriate responding. Tests of stimulus generalization (substitution) have been conducted to determine the similarity of effects produced by a test drug to those produced by the training dose of nicotine. Such tests have shown that other "natural" tobacco alkaloids and metabolites of (−)-nicotine can produce nicotine-like effects, but these drugs are less potent than (−)-nicotine. Stimulus antagonism (blockade) tests confirm that the (−)-nicotine stimulus is mediated via brain α4β2 nAChR subtype receptors. This conclusion is based on reports that nicotine stimuli are blocked by (a) mecamylamine, a noncompetitive channel blocker at nAChRs and (b) dihydro-β-erythrodine, a nicotinic α4β2 subunit receptor antagonist, but not by (c) methyllycaconitine, an α7 nAChR receptor antagonist. In other studies, (−)-nicotine stimuli appear to share a marked degree of effects with bupropion (Zyban®) and varenicline (Chantix®), pharmacotherapies prescribed for smoking cessation. However, studies have not been conducted with the latter two drugs as training drugs to assess the effects of (−)-nicotine in stimulus generalization tests. Results from such studies would determine if cross generalization occurs between the drugs and could elucidate more clearly the relationships between the stimulus effects of (−)-nicotine versus those of the prescribed treatment medications. Overall, the application of drug discrimination procedures to study the effects of (−)-nicotine has achieved much success and progress. At this point in time, however, the model should be directed and applied to the issue of maintenance of abstinence from nicotine-based products. Thus, when an individual tries to quit nicotine consumption, cessation of use is typically followed by a withdrawal period that, unfortunately, usually leads to relapse; withdrawal symptoms include anxiety, depression, craving, cognitive and attention deficits. Moreover, just about every form of nicotine cessation therapy that has been employed typically demonstrates high immediate success rates, but high relapse rates almost certainly follow. The discriminative stimulus model demonstrates specificity and strength. These attributes could prove useful in the invention of new pharmacotherapies to assist the individual who desires to end their use of nicotine. For example, Harris et al. [219] trained rats to discriminate pentylenetetrazol [a.k.a. metrazol (PTZ)] from saline and suggested that the basis for the discrimination was PTZ-induced anxiety. In support of this argument, they reported that their animals showed a PTZ-like response when they were in nicotine "withdrawal." That is, their

PTZ-trained rats were administered high doses of nicotine over a 3-week period and subsequently responded on the PTZ-appropriate lever 24 h after the cessation of dosing. These investigators suggested that rats in nicotine withdrawal may be experiencing "anxiety" as measured by their PTZ partial generalization response. This application of the model could be an important finding: the potential to measure subjective effects during withdrawal from (−)-nicotine. Follow-up studies could exploit the finding and evaluate targeted drugs as potential antagonists of the PTZ-like withdrawal response. This approach may be able to identify candidate drugs to assist people who want to cease their consumption of (−)-nicotine-based products.

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Cross-Species Translational Findings in the Discriminative Stimulus Effects of Ethanol

Daicia C. Allen, Matthew M. Ford, and Kathleen A. Grant

Abstract The progress on understanding the pharmacological basis of ethanol's discriminative stimulus effects has been substantial, but appears to have plateaued in the past decade. Further, the cross-species translational efforts are clear in laboratory animals, but have been minimal in human subject studies. Research findings clearly demonstrate that ethanol produces a compound stimulus with primary activity through GABA and glutamate receptor systems, particularly ionotropic receptors, with additional contribution from serotonergic mechanisms. Further progress should capitalize on chemogenetic and optogenetic techniques in laboratory animals to identify the neural circuitry involved in mediating the discriminative stimulus effects of ethanol. These infrahuman studies can be guided by in vivo imaging of human brain circuitry mediating ethanol's subjective effects. Ultimately, identifying receptors systems, as well as where they are located within brain circuitry, will transform the use of drug discrimination procedures to help identify possible treatment or prevention strategies for alcohol use disorder.

Keywords Alcohol • Drug discrimination • Ethanol • Interspecies • Translational

D.C. Allen
Department of Behavioral Neurosciences, Oregon Health & Science University, Portland, OR 97239, USA

M.M. Ford and K.A. Grant (✉)
Department of Behavioral Neurosciences, Oregon Health & Science University, Portland, OR 97239, USA

Division of Neuroscience, Oregon National Primate Research Center, Beaverton, OR 97006, USA
e-mail: grantka@ohsu.edu

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Contents

1 Introduction

Although reports of state dependent learning using alcohol go back to the 1950s [1], the study of the pharmacological basis of the ethanol discriminative stimulus began in earnest in the early 1970s. The literature reviewed here comprises reports in which ethanol was used as a training stimulus as well as manuscripts that used ethanol in substitution tests for other drugs used as training stimuli. Studies that report only subjective effects or reinstatement procedures are not included in this review. In general, given that the cumulative dataset encompasses over four decades of research, the volume of studies addressing the discriminative stimulus effects of ethanol is not large. Figure 1 depicts both the cumulative publications (Fig. 1a) and the yearly publication rate (Fig. 1b) reporting ethanol trained as a discriminative stimulus as well as ethanol substitution tests in other drug discriminations. Most of the substitution tests using ethanol were either to control for nonspecific drug effects (i.e., as a negative control for discriminative stimuli other than ethanol) or to test for cross-generalization. These contributions have been at a low, but fairly consistent rate over time (Fig. 1b). Overall, for studies that trained an ethanol discrimination there has been about 4–5 publications/year, but the timeframe of 1990–2005 was clearly the most productive (Fig. 1a). Publications have fallen off considerably since 2005. This trend is remarkably similar to the trend encompassing the entire drug discrimination literature as recently reviewed [2]. The reason for this decline in the use of ethanol discrimination in understanding the behavioral pharmacology of ethanol is not readily obvious. Given the utility of drug discrimination as an in vivo pharmacological assay, there clearly remain many important questions that can be addressed with an ethanol discrimination preparation, particularly in the context of recent advancements in brain-region specific targeting and genetic manipulations. Neurobiological approaches, including chemogenetic and optogenetic manipulations, have the potential to greatly expand our knowledge of dose-dependent mechanisms of ethanol in the brain and improve cross-species translational cohesion of the interoceptive effects of ethanol. In general, animal models of human behavior ultimately strive to provide data that inform the human condition. In alcohol discrimination procedures, this emphasis is on pharmacological variables and receptor mechanisms that mediate the stimulus effects and a rational approach to pharmacotherapeutic development for alcohol use disorders. However, specificity in terms of

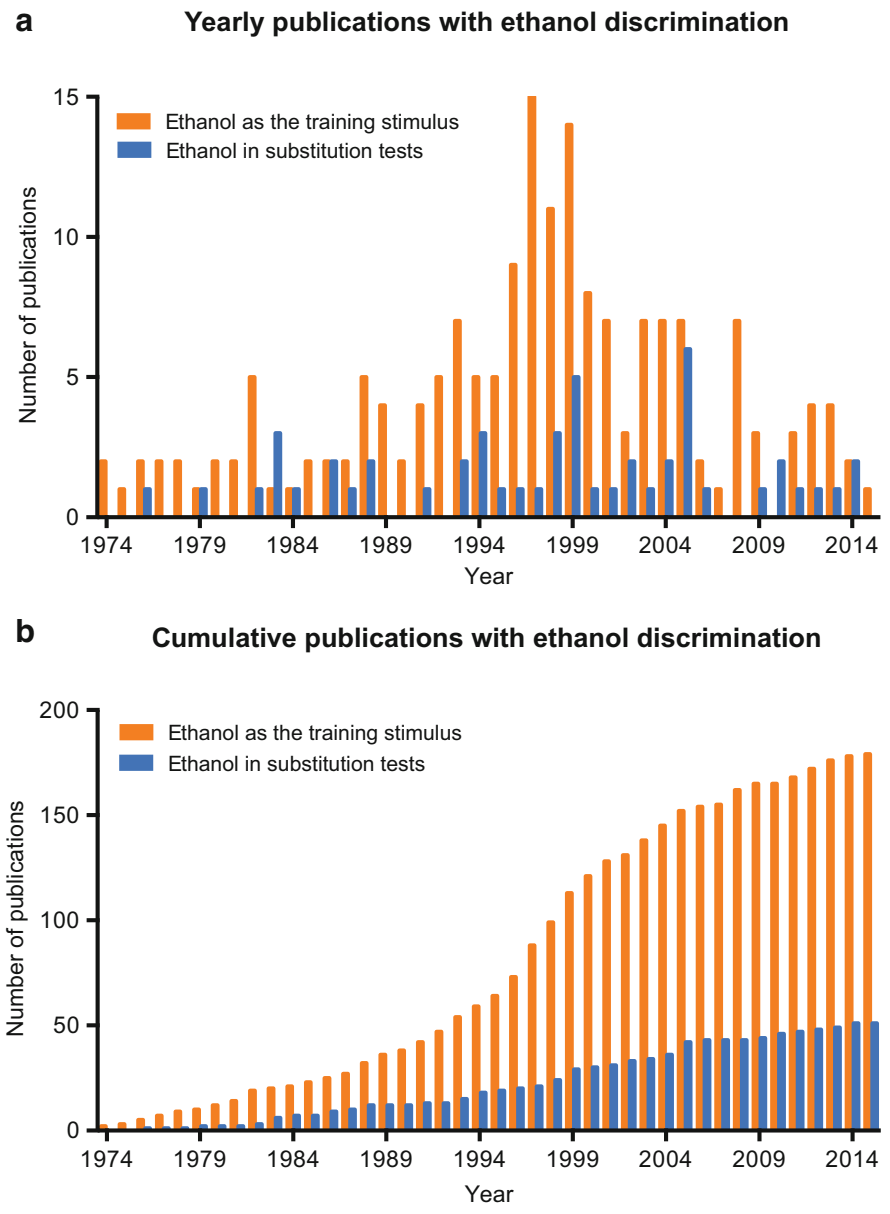

Fig. 1 Cumulative (**a**) and yearly (**b**) publication rate for ethanol as a training stimulus and in substitution tests

discrete neural circuitry to target and lower adverse off target effects has not been achieved, but represents an important avenue forward.

Although circuitry and genomic approaches are only just beginning to be applied to ethanol discriminations, ethanol is one substance that has a relatively strong record in cross-species translational studies. Ethanol has been trained as a discriminative stimulus in pigeons, mice, rats, gerbils, monkeys, and humans, although not in a proportional fashion. Indeed, the contribution to the literature can be rank ordered by rodents (89%), monkeys (7%), pigeons (2%), and humans (2%). Somewhat perplexing, given the legal and historical status of alcohol, there are far fewer human subject studies of ethanol discrimination than there are of stimulant, opiate, or cannabinoid discriminations (reviewed in Bolin et al. [2] and in the section below).

Given the low rate of new data on ethanol discrimination in recent years, an in-depth review of the basic pharmacology underlying ethanol's discriminative stimulus effects would add little to available reviews on the subject [3–6]. Instead, this review focuses on more recent developments in refining the specific action of ethanol at each receptor target. Specifically, the receptor systems that are primarily identified in rodents will be compared and contrasted with findings from monkeys and humans in order to highlight whether this information has been translated across species. A major conclusion is that in order for ethanol discrimination studies to advance pharmacotherapies for alcohol use disorders, a new approach is needed. Specifically, the future of translational ethanol discrimination studies must focus on region specific receptor mechanisms and how this fits into a cohesive understanding of brain circuitry. Over the last four decades, ethanol drug discrimination studies have established three primary receptor targets involved in ethanol's discriminative stimulus effects: $GABA_A$, NMDA, and $5\text{-}HT_{1B/2C}$ systems. There has also been some evidence for a secondary, modulatory role of both the opioid [7–11] and acetylcholine [12–16] receptor systems, but there is no evidence of direct mediation of ethanol's discriminative stimulus effects at these receptor sites. Ethanol is known to act as a positive modulator at the $GABA_A$ receptor to increase chloride conductance through the channel and decrease cellular excitability [17]. Additionally, ethanol has antagonist activity at the NMDA glutamate receptor, which appears selective for noncompetitive antagonism. Lastly, ethanol has activity at several 5-HT receptor systems, but agonism at the $5\text{-}HT_{1B/2C}$ receptor subtypes is most prominent [4, 6, 18].

Somewhat unique to ethanol, the relative contribution of these stimulus components varies based on training dose magnitude, with $GABA_A$ receptors exerting greatest influence at low to moderate training doses ($\leq$1.5 g/kg) and NMDA receptors playing a larger role at higher doses ($\geq$1.5 g/kg) in rodents [6, 19, 20]. Similarly, the 5-HT component of the ethanol stimulus complex is most prominent at low to moderate training doses [21]. More recent work expands upon this foundation and emphasizes the selectivity of ethanol at different receptor subtypes and subunits by incorporating novel ligands. To compare data across species, findings from systemic administration are surveyed in the following sections, with an additional section emphasizing recent work with targeted brain-region approaches. At the conclusion, suggestions for future approaches are presented to maximize the utility of ethanol discrimination procedures for pharmacotherapy development.

1.1 Rodents

1.1.1 GABA

The $GABA_A$ receptor complex is integral to many of ethanol's behavioral and physiological effects (e.g., [17, 22]). Consistent with ethanol's action as a positive modulator at the $GABA_A$ receptor, drugs in the benzodiazepine and barbiturate classes, with a similar mechanism to modulate chloride flow through the $GABA_A$ receptor, consistently produce ethanol-like discriminative stimulus effects (reviewed in Grant [4]). More recent work has expanded upon these findings in two primary ways. First, the specific action of ethanol at $GABA_A$ receptors with distinct subunit compositions has been investigated using a combination of genetic knockout and selective ligand approaches. Second, the selective role of neurosteroid activity at the $GABA_A$ receptor has been confirmed, and consistent with the action of neurosteroids as positive allosteric modulators at $GABA_A$, they exhibit ethanol-like discriminative stimulus effects similar to those generated by the benzodiazepine and barbiturate drug classes.

The $GABA_A$ receptor is a pentameric transmembrane receptor, classically made up of two alpha (α) subunits, two beta (β) subunits, and one gamma (γ) subunit. A delta (δ) subunit may substitute for a γ subunit in some receptor isoforms. Ethanol discrimination studies have primarily focused on isolating the role of α1-, α4/6-, and δ-subunit-containing receptors. Specifically, zolpidem, an α1 subunit-preferring benzodiazepine agonist, partially substitutes for ethanol in rats [23], but does not produce ethanol-like stimulus effects in mice [24], suggesting that activity at the α1 subunit is not sufficient to produce ethanol discriminative stimulus effects in rodents. Additionally, ethanol's action at α4/6-subunits has been investigated using Ro 15-4513, an inverse agonist at the benzodiazepine binding site, with some selectivity for the α4/6-subunits. While Ro 15-4513 successfully antagonizes the discriminative stimulus effects of benzodiazepine, the results are mixed for ethanol-trained rodents, with some studies showing antagonism of ethanol's discriminative effects [25, 26], and others showing no antagonism [27, 28]. The mixed effects of Ro 15-4513 as an ethanol antagonist are likely due to the differences in training doses and routes, suggesting that the prominence of the α4/6-subunits in ethanol discrimination is dependent on experimental parameters that might influence BEC. The δ-subunit of the $GABA_A$ receptor complex has also been isolated in ethanol discrimination using a constitutive δ-subunit knockout line of mice, and the results indicated that there were no differences in either the acquisition of ethanol discrimination or the substitution patterns of the $GABA_A$ receptor positive modulators compared to wild-type mice [24]. Therefore the δ-subunit of $GABA_A$ receptors is not necessary for mediating ethanol-like discriminative stimulus effects or for the substitution of benzodiazepines, barbiturates, or neurosteroids. The δ-subunit is thought to be an identifying feature of extrasynaptic $GABA_A$ receptors that mediate tonic inhibitory currents and confer sensitivity to low doses of ethanol [29, 30], and thus, these findings suggest that either non-δ extrasynaptic or synaptic receptors associated with phasic inhibitory currents may be more prominent in producing the discriminative stimulus effects of ethanol.

The steroid binding site on $GABA_A$ receptors and its modulation by neuroactive steroids has received considerable attention because these endogenous compounds respond to stress and are implicated in a number of behavioral disorders [31]. Neuroactive steroids that act at $GABA_A$ receptors do so through binding sites that are distinct from the benzodiazepine and barbiturate sites, and the conformation of the steroid A-ring 3′ and 5′ carbon hydroxyl groups is the key to receptor activation (see Chen et al. [32]). Select neuroactive steroids generalize from an ethanol training stimulus in rodents, including the reduced metabolites of progesterone (allopregnanolone or 3α,5α-P; pregnanolone or 3α,5β-P; and epipregnanolone or 3β,5β-P) and deoxycorticosterone (allotetrahydro-deoxycorticosterone or 3α,5α-THDOC) [33, 34]. Substitution was more prominent at a lower training dose (1 g/kg, i.g.) versus a higher one (2 g/kg, i.g.) [34]. The ethanol route of administration may also play a role in substitution patterns as 3β,5β-P has mixed effects in ethanol discriminations. 3β,5β-P produced no generalization with ethanol trained via an intraperitoneal route [34] but produced complete substitution, as well as potentiation of the ethanol cue, when trained with an intragastric route [33, 35]. Finally, the neurosteroid substitution patterns for ethanol suggest sex differences in sensitivity. For example, in contrast to earlier studies in male rats [33, 34], female rats showed only partial substitution of allopregnanolone and pregnanolone for a 1 g/kg ethanol training dose [36]. This latter finding is consistent with earlier work demonstrating that females were less sensitive to the modulatory effects of allopregnanolone on ethanol drinking behavior when compared to males [37]. Collectively, these and other studies (e.g., [38]) suggest that $GABA_A$ receptors that contain a neurosteroid binding site contribute to the discriminative stimulus effects of ethanol. Similar to barbiturates and benzodiazepines, neuroactive steroids asymmetrically cross-generalize with ethanol, with only partial substitution when ethanol is substituted in pregnanolone-trained rats [39–41] and mice [42]. This asymmetrical cross-generalization likely reflects the inability of pregnanolone and related neuroactive steroids to encompass other aspects of the compound ethanol cue.

1.1.2 Glutamate

The NMDA glutamatergic receptor is also well established in contributing to the discriminative stimulus effects of ethanol, particularly at higher doses in rodents [8]. Consistent with ethanol's known action as an NMDA antagonist at the synapse [17], drug discrimination studies have established that antagonism of the NMDA receptor produces ethanol-like discriminative effects. One of the earliest studies determined that the noncompetitive channel blocker dizocilpine (i.e., MK-801) fully substituted for ethanol in pigeons [43], and this finding has been replicated in rodents, including multiple strains of rats [19, 44–48] and mice [24, 49]. Other NMDA channel blockers such as memantine, phencyclidine (PCP), and ketamine have yielded similar degrees of substitution for ethanol in rats [19, 45, 47]. Often, however, substitution requires doses of the NMDA antagonists that also attenuate response rates [44, 50] to the extent that full substitution by these compounds is precluded [51].

In addition to the channel blocker site, multiple binding sites on the NMDA receptor have been examined, including the glutamate, glycine, and polyamine sites. Overall, ligands for each of these other binding sites have been far less effective in producing ethanol-like stimulus effects, indicating that ethanol's action is most similar to the non-competitive activity at the channel pore. Competitive antagonists at the glutamate site have generalized from ethanol in some cases (CGS 19755) [47], but have only partially substituted in other cases (CPPene, NPC-17742) [44, 51]. Similar results have been found with glycine site antagonists, with some ligands producing full substitution (L701,324) [50, 52], and others not substituting at all (MRZ2-502 and MRZ2-576) [45, 50]. Lastly, polyamine binding site antagonists (eliprodil and arcaine) produce stimulus effects that do not generalize from ethanol [45, 47]. In conclusion, the contribution of the glutamate, glycine, and polyamine binding sites of the NMDA receptor appears minimal in ethanol discrimination, particularly when compared to the channel pore site. However, it is noteworthy that aforementioned studies were all conducted in rats trained to discriminate a low to moderate dose of ethanol (i.e., 1 g/kg), and it is possible that inconsistent findings between studies may be partially attributable to the training dose studied, as previous work indicates that NMDA receptors contribute more predominantly to the ethanol stimulus at higher doses (>1.5 g/kg) in rodents [6, 19, 52].

In addition to the NMDA receptor, recent studies have begun to examine the metabotropic glutamate system (mGluR1, mGluR2/3, and mGluR5) based on findings that the mGluR5 receptor might modulate activity at the $GABA_A$ receptor [53]. Selective mGluR5 antagonist MPEP antagonized the ethanol dose–response function by decreasing the potency for ethanol to substitute for itself [53–55]. An mGluR2/3 agonist also decreased the potency of ethanol discrimination [56], but no effect was observed with any of the mGluR1 antagonists tested [54]. These studies have provided a novel pharmacological target for ethanol's discriminative stimulus effects, although it should be noted that these effects are modulatory in nature, and they are not sufficient to produce ethanol-like effects on their own. Thus, the direct glutamatergic activity of ethanol remains primarily at the NMDA receptor.

1.1.3 Serotonin

The importance of serotonergic neurotransmission in ethanol discriminative stimulus effects was first reported with the observation that pretreatment with a tryptophan hydroxylase inhibitor (p-chlorophenylalanine; which depletes brain 5-HT) reduces compartment choice between ethanol and water to chance levels in rats studied within a shock avoidance-based discrimination paradigm [57]. Since then, there have been several studies to manipulate levels of synaptic 5-HT, through enhancing 5-HT release (fenfluramine), a nonselective 5-HT receptor agonist (5-MeODMT), and selective serotonin uptake inhibitors (SSRIs; fluoxetine and paroxetine). In general, only SSRIs have produced ethanol-like discriminative stimulus effects [58], but this may be mediated through a non-serotonergic mechanism via their augmentation of brain allopregnanolone levels [59], which would be expected to exert positive modulation of $GABA_A$ receptors.

The first 5-HT receptor to be examined in an ethanol discrimination preparation was the 5-HT_3 receptor [60], which is an ionotropic receptor, and therefore from the same superfamily of receptors as the $GABA_A$ and NMDA receptors. Although studies in rats have found that 5-HT_3 receptor agonists (mCPBG) and antagonists (ICS 205-930) do not generalize from ethanol [61, 62], there is some limited evidence in pigeons that 5-HT_3 receptor antagonists (ICS 205-930 and MDL 72222) block the discriminative stimulus effects of low to moderate ethanol doses [63]. These data suggest that contribution of 5-HT_3 receptors in producing discriminative stimulus effects of ethanol is likely minimal. This conclusion is also supported by data from transgenic mice that overexpress 5-HT_3 receptors and show no differences in their ability to acquire an ethanol discrimination or in the substitution profiles with $GABA_A$ receptor positive modulators and an NMDA receptor antagonist when compared to wild-type mice [64].

In contrast to nonselective or selective 5-HT_3 receptor agonists, there is sufficient evidence to indicate a role for agonism at metabotropic 5-HT receptor subtypes in ethanol discrimination. From an initial characterization of several 5-HT receptor agonists in rats, the only compound to yield full substitution for ethanol in rats was TFMPP, a relatively nonselective 5-HT_1 agonist with slightly greater affinity for the 1A isoform [65]. This finding with TFMPP was replicated in both male [21] and female [36] rats. Subsequent evaluations of multiple compounds with various 5HT receptor agonist profiles in male rats revealed that CGS 12066B and CP 94,253 (both selective for 5-HT_{1B}) or mCPP and RU 24969 (both selective for 5-$HT_{1B/2C}$) fully generalized from ethanol (1 g/kg), whereas 8-OH DPAT (5-HT_{1A}) and DOI (5-HT_{2A}) did not [66–68]. A parallel set of antagonism studies used subtype selective antagonists to completely block the ethanol-like effects of CP 94,253 and mCPP [67], leading to an overall conclusion that 5-HT_{1B} and 5-HT_{2C} receptors contribute to the ethanol cue. However, there are inconsistencies in the generalizability of 5-$HT_{1B/2C}$ agonists to substitute for ethanol across sex and species, as RU 24969 only partially substituted for ethanol in female rats [36] and mCPP did not generalize from ethanol in mice [64]. Refinement of receptor ligands with increased selectivity for 5-HT_1 and 5-HT_2 receptor isoforms (e.g., [69, 70]) coupled with a rapid expansion of novel ligand development for 5-HT_4 receptors, which also function to regulate neurotransmission in conjunction with 5-HT_1 and 5-HT_2 receptors [71, 72], should prompt a fresh look at the involvement of metabotropic 5-HT receptors in modulating the discriminative stimulus effects of ethanol.

1.2 Nonhuman Primates

Ethanol discrimination in monkeys has built upon findings from rodents in several key ways. In general, nearly all of the receptor targets of ethanol in monkeys have been taken from the rodent literature and are largely consistent across species. However, there are several important differences between the rodent and the monkey that may inform future clinical work and shed light on potential limitations of smaller laboratory animals in ethanol discrimination. Nonhuman primate studies have primarily

focused on ethanol's action at the $GABA_A$ and NMDA receptors, with some work on the opioid system. Additionally, nonhuman primate work has examined other biological variables that may contribute to ethanol's discriminative stimulus effects, such as sex [73–76], age [77], and menstrual cycle [78].

Ethanol's action at the $GABA_A$ receptor is highly selective in nonhuman primates. Specifically, studies in monkeys have examined subunit-selective ligands and antagonists at the $GABA_A$ receptor [75, 79–81], as well as neuroactive steroid activity [74, 78, 82, 83]. Additionally, cross-generalization analysis was possible by studies that trained ethanol-like $GABA_A$ ligands and examined ethanol in substitution tests [79, 84–86]. Similar to rodents, direct agonists at the $GABA_A$ receptor fail to produce ethanol discriminative stimulus effects, but positive allosteric modulators reliably substitute for ethanol [73]. Specifically, positive modulators at the benzodiazepine and barbiturate binding sites produce the most robust ethanol-like effects [73]. In contrast to rodents, however, $GABA_A$ modulators produce full substitution at low and high training doses (1.0–2.0 g/kg), rather than just predominantly at lower doses. Converging evidence from multiple studies suggests that α5 subunit-containing receptors are particularly important in ethanol's discriminative stimulus effects [75, 80, 81], as well as some contribution of the α1 and α2/3 subunits. Alpha-5 and alpha-1 selective agonists substitute for ethanol, but only inverse agonists selective for α5 (L-655,708) and α5 + α4/6 (Ro-154513) are able to antagonize ethanol's discriminative stimulus effects [75, 87]. Ro-154513 is also able to antagonize the substitution of benzodiazepines and barbiturates for ethanol, suggesting a shared action at the $GABA_A$ subunit level [76]. Neuroactive steroids also selectively produce ethanol-like discriminative effects based on their pharmacological effect at the $GABA_A$ receptor. Specifically, 3-alpha-hydroxy metabolites of progesterone such as allopregnanolone and pregnanolone are positive modulators at the $GABA_A$ receptor and produce ethanol-like stimulus effects in male and female monkeys [74, 82, 83]. However, 3-beta-hydroxy metabolites do not reliably substitute for ethanol at any training dose [80]. Several studies in monkeys have trained $GABA_A$ ligands and tested ethanol for substitution. To summarize this work, ethanol only cross-substituted with pentobarbital [85], but did not substitute for midazolam [86] or lorazepam [84]. These data suggest that ethanol's discriminative stimulus effects in the monkey are more similar to barbiturates, as compared to benzodiazepines.

Ethanol's discriminative stimulus effects are also mediated by antagonist activity at the NMDA receptor, and may be modulated by the opioid system. Noncompetitive antagonists at the channel pore MK-801 (or dizocilpine) and PCP produce full substitution for ethanol in male and female monkeys, but (unlike rodents) ketamine has not produced full substitution [76]. NMDA antagonist substitution was most potent and efficacious at a lower training dose, which is also in contrast to studies in rodents suggesting that a higher ethanol training dose conferred greater NMDA antagonism substitution [6] (see Sect. 1.1 above). These data are consistent with rodent data in characterizing ethanol as a compound stimulus in the monkey, with activity at both $GABA_A$ and NMDA receptors. Further, there has been a limited attempt to characterize the role of mu and delta opioid receptors in mediating the ethanol cue in monkeys. This examination found that selective agonists at both the mu (i.e., morphine and fentanyl) and delta (i.e., SNC 80 and SNC 162) receptors did not produce ethanol-like

stimulus effects [73, 87], indicating that the opioid system is likely not a primary target in ethanol's discriminative stimulus effects. However, nonselective antagonist naltrexone antagonized the ethanol dose–response relationship [87], suggesting that the opioid system may function as a modulator of the ethanol stimulus, adding to the complex basis of the ethanol cue.

Lastly, nonhuman primate studies have taken advantage of the overlapping physiology between humans and monkeys to examine biological variables that may contribute to ethanol's discriminative stimulus effects. Most notably, a few of the nonhuman primate studies have directly compared male and female subjects in the analysis of $GABA_A$ and NMDA receptor involvement in ethanol's discriminative stimulus effects [73, 76]. Though there are small differences between male and female monkeys, in general the pharmacological basis of the ethanol cue is shared across the sexes. One exception relates to neurosteroid substitution for ethanol, which appears dependent on the phase of the menstrual cycle in female monkeys [78, 83]. In the luteal phase, when progesterone levels are high, allopregnanolone is more potent in its substitution for ethanol, consistent with greater levels of allopregnanolone in the plasma. Lastly, one study examined the effect of age on ethanol discriminative stimulus effects and determined that ethanol served as a relatively weaker stimulus in middle-aged monkeys, despite elevated blood ethanol concentrations relative to when the same monkeys were young adults [77]. Additionally, this study demonstrated that ethanol discrimination was persistent and demonstrated up to 3 years without any intermediate training [77].

1.3 Humans

To our knowledge, there are only five reports of training ethanol as a discriminative stimulus in human subjects [88–92] and one report of ethanol substitution in a nicotine-trained discrimination, in which it did not substitute [93]. These studies primarily demonstrated that ethanol can be trained with equal sensitivity in male and female subjects [88, 91], but the acquisition is sensitive to baseline weekly alcohol intake [89, 90] and ethanol generalization occurs in a dose-dependent manner [88, 89, 92]. The only study to test a compound other than ethanol examined the benzodiazepine lorazepam and found complete substitution [91]. Thus, the only receptor system directly implicated in the basis of an ethanol discrimination in humans is the $GABA_A$ receptor system.

1.4 Neuroanatomical Targets

In the last 20 years, there have been a handful of laboratories that have investigated the neuroanatomical basis of ethanol's discriminative stimulus effects. These studies have been conducted exclusively in rodents and have focused on the GABA and glutamate components of the ethanol cue using intracranial site-specific microinjections. Additionally, some work has been done measuring c-Fos activation after

performance of an ethanol discrimination to identify the primary brain regions involved in ethanol's discriminative stimulus effects and the direction (activation or inactivation) of their involvement. A majority of these studies are based on an initial finding that agonism of the $GABA_A$ receptor in the nucleus accumbens (NAc) produced full substitution for ethanol [94]. Since then, $GABA_A$ positive modulators such as pentobarbital and allopregnanolone administered into the NAc core have also produced full ethanol substitution [94–96]. However, ethanol substitution is not blocked by the $GABA_A$ antagonist bicuculline in the NAc indicating that $GABA_A$ receptors within the NAc are sufficient, but not necessary to produce ethanol-like discriminative stimulus effects [94]. This is supported by work demonstrating that NMDA antagonist MK-801 in the NAc also produces full substitution for ethanol [96], and there appears to be some secondary contribution of mGlu5 receptors in the NAc, consistent with systemic administration of these compounds [54]. Thus, it appears that within the NAc, ethanol is acting as a compound cue on GABA and glutamate systems. It is important to note that these findings are highly consistent with ethanol's known action to activate $GABA_A$ and inhibit NMDA activity within the NAc in slice electrophysiology studies [17, 97, 98], resulting in an overall suppression of neuronal firing. This is further supported by c-Fos studies in discrimination-trained rats demonstrating decreased c-Fos activity within the NAc after ethanol [99, 100].

In addition to the NAc, there have also been select studies examining the role of the amygdala, several cortical areas (mPFC, prelimbic, and insula), hippocampus, and thalamus (rhomboid nucleus). In general, these primarily limbic brain regions have been demonstrated to contribute to some extent to ethanol's discriminative stimulus effects. Interestingly, these brain areas appear to have some selectivity for whether they are involved primarily in ethanol's GABAergic or glutamatergic component. Specifically, $GABA_A$ modulation in the amygdala produces ethanol-like effects, but there is no evidence for this brain region in the NMDA component [96, 101]. Conversely, NMDA the antagonist MK-801 in the prelimbic cortex and hippocampus produced full ethanol substitution, but $GABA_A$ agonists did not substitute [96]. The mPFC, insula, and rhomboid thalamus have also been shown to contribute to the GABA component through pharmacological inactivation using a $GABA_A + GABA_B$ cocktail [100]. This fairly limited body of literature raises some important questions that can be addressed with future research. A differential contribution of different brain structures to the compound ethanol cue strongly suggests that our focus should be redirected to understanding sensitive circuitry mediating the discriminative stimulus effects of ethanol. Because the preliminary data on sensitive brain areas (not circuitry per se) is exclusively derived in rodent subjects, replicating and extending these results to the primate brain is needed.

1.5 New Paradigm for Advancing Knowledge and Pharmacotherapeutic Development with Ethanol Discriminations

From a translational perspective, these brain circuitry studies in rodents provide a strong foundation for potential target sites for future work in monkeys and humans. The recent development of chemogenetic or optogenetic approaches, using viral-based molecular targeting strategies, will allow for repeated manipulation of specific brain nuclei to understand their role in mediating the ethanol cue. Additionally, application of fMRI techniques in humans can examine the connectivity patterns of brain activation in mediating the discriminative stimulus effects of ethanol. The combination of human brain mapping and functional testing of identified areas in animal models with molecular targeting approaches will open up a new understanding of how the subjective effects of ethanol are mediated. Overall, although the number of laboratories involved in ethanol discrimination studies appears to be declining, these new technologies are likely to revive interest in knowing how the ethanol cue is mediated and its role in the subjective effects that maintain human alcohol consumption.

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Discriminative Stimulus Effects of Abused Inhalants

Keith L. Shelton

Abstract Inhalants are a loosely organized category of abused compounds defined entirely by their common route of administration. Inhalants include volatile solvents, fuels, volatile anesthetics, gasses, and liquefied refrigerants, among others. They are ubiquitous in modern society as ingredients in a wide variety of household, commercial, and medical products. Persons of all ages abuse inhalants but the highest prevalence of abuse is in younger adolescents. Although inhalants have been shown to act upon a host of neurotransmitter receptors, the stimulus effects of the few inhalants which have been trained or tested in drug discrimination procedures suggest that their discriminative stimulus properties are mediated by a few key neurotransmitter receptor systems. Abused volatile solvent inhalants have stimulus effects that are similar to a select group of $GABA_A$ positive modulators comprised of benzodiazepines and barbiturates. In contrast the stimulus effects of nitrous oxide gas appear to be at least partially mediated by uncompetitive antagonism of NMDA receptors. Finally, volatile anesthetic inhalants have stimulus effects in common with both $GABA_A$ positive modulators as well as competitive NMDA antagonists. In addition to a review of the pharmacology underlying the stimulus effects of inhalants, the chapter also discusses the scientific value of utilizing drug discrimination as a means of functionally grouping inhalants according to their abuse-related pharmacological properties.

Keywords 1,1,1-trichloroethane • Abuse • Drug discrimination • Inhalant • Isoflurane • Nitrous oxide • Toluene • Trichloroethylene • Volatile vapor

K.L. Shelton (✉)
Department of Pharmacology and Toxicology, Virginia Commonwealth University, 410 North 12th Street, Room 746, P.O. Box 980613, Richmond, VA 23298-0613, USA
e-mail: klshelto@vcu.edu

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Contents

1 Introduction

Inhalant abuse is a major worldwide public health problem. In the USA approximately one million adults, 18 years and older, used an inhalant in 2013 (SAMSA). This is roughly equal to the reported number of methamphetamine users and almost twice that reported to have used heroin. A number of other drugs including marijuana, prescription drugs, and cocaine are abused at higher rates than inhalants in adults. However, in younger adolescents, inhalant abuse is far more prevalent. Lifetime use of inhalants in 8th graders in 2014 was estimated at 10.8%, which ranked below only marijuana among illicit drugs [1]. The demographics of inhalant abuse are particularly troubling given the possibility that repeated inhalant exposure during the vulnerable adolescent development period may have long-lasting effects, which may not be immediately apparent. Chronic inhalant abuse can produce profound toxic effects to many organs including the liver, bone marrow, heart, and brain [2–5]. These risks and other factors make understanding the neurochemical effects underlying the abuse-related effects of inhalants an important priority. Unfortunately several challenges complicate this task, some of which may be overcome through the use of drug-discrimination procedures.

Inhalants stand alone as being the only major classification of abused drugs that is based solely on a shared route of abuse rather than established similarities in pharmacological actions. Most inhalants are volatile liquids possessing vapor pressures that permit them to readily form vapors at room temperature. Also included are products such as propane and butane as well as various liquefied refrigerants that are compressed into a liquid form which exist as gasses at atmospheric pressure. Finally, the anesthetic adjunct gas nitrous oxide is also considered an inhalant within the drug abuse research community.

Many inhalants are consumer or industrial products comprised of mixtures of various volatile compounds, which may or may not share common pharmacological properties. For instance, gasoline is a mixture composed of toluene, hexane, xylene, octane, and ethanol along with perhaps a dozen additional minor constituents. This

chemical complexity makes studies on the abuse-related effects of consumer products problematic. Therefore, almost all research has focused on a small number of individual chemicals commonly present in abused consumer products [6, 7]. Among these, the aromatic hydrocarbon toluene likely has the highest abuse rate [8]. Toluene is present in gasoline, pain thinners, wood coloring stains, spray paints, and cleaning products. The addictive nature of toluene is strikingly illustrated by the precipitous drop in gasoline abuse in areas of Australia where a toluene-free gasoline was introduced by BP to combat rampant abuse [9]. Unfortunately, other commonly abused volatile and gaseous chemicals have received far less attention in the scientific literature than toluene.

Given limited resources a complete understanding of the abuse-related effects of every common inhalant is unrealistic. Instead a more reasonable goal might be to thoroughly explore the actions of a lesser number of inhalants that are known to have differing pharmacological actions. These inhalants could then serve as reference standards with which to compare others. However, it has yet to be adequately established whether pharmacologically distinct subgroups of inhalants even exist. This is one area in which the use of drug discrimination may be particularly helpful given the ability of drug discrimination to compare and contrast drugs using a relevant, abuse-related endpoint.

In the absence of sufficient data to permit inhalants to be segregated by pharmacological activity, other proposed classification systems of inhalants have been suggested [10]. For instance, many inhalants are volatile hydrocarbon chemicals with common structural characteristics. Examples include aromatic hydrocarbons (toluene, xylene), chlorinated hydrocarbons (1,1,1-trichloroethane, trichloroethylene), and halogenated hydrocarbons (isoflurane, sevoflurane). At this time the classification of inhalants according to chemical structure has limited pharmacological value as the importance structural characteristics which determine pharmacological mechanisms of action of inhalants have not been defined as has been the case with some other classes of drugs [11, 12]. Therefore, the most widely accepted system of inhalant classification at present is to group them according to their intended usage. The scientific consensus has generally chosen to utilize the three categories proposed by Balster et al. [10] and Balster [13]. These categories include (1) volatile solvents, fuels, and anesthetics; (2) volatile alkyl nitrites; and (3) nitrous oxide. However, as with any framework some entities are not easily categorized. For instance, liquefied compressed refrigerants such as chloroethane and 1,1,1,2-tetrafluoroethane (R134a) do not fit into any of these categories. Broadly speaking, inhalants grouped according to the categories proposed by Balster do appear to bear some similarities in observable behavioral effects but the categories are probably too inclusive to serve as a reliable indicator of pharmacological actions. The system has, however, proven helpful as an interim solution to a challenging problem.

2 Unique Methodological Aspects of Inhalant Discrimination Studies

The technical issues related to drug-discrimination studies with inhalants have been discussed in greater detail elsewhere and will only be touched upon here (for review see [14]). Briefly, methods previously developed to produce consistent and reproducible inhalant exposures to examine other endpoints have been adapted for use in drug discrimination, the most common being the static exposure procedure. The exposures are "static" in the sense that a test subject and a measured volume of a volatile liquid inhalant chemical are confined in a sealed chamber of a fixed volume for a specified period of time. Provided the liquid inhalant can be completely volatilized, the ideal gas law is used to calculate inhalant chamber concentration without resorting to complicated quantitative assessment methods.

Static exposure chamber concentration and exposure duration are in most respects analogous to drug dose and pretreatment time in traditional drug discrimination studies. However, unlike injected drugs, after the cessation of exposure inhalant blood levels immediately begin to decline as a result of elimination via exhalation. This results in pharmacological effects that are quite labile compared to drugs administered by other routes. Under common drug discrimination training exposure conditions designed to mimic the short duration of inhalation typical of abuse, the majority of toluene within the bloodstream is eliminated unchanged by exhalation with very little undergoing metabolism. In mice trained to discriminate 10 min exposure to 6,000 ppm toluene vapor from 10 min exposure to air, toluene-lever selection declined to less than 50% within 10 min after the cessation of exposure [15] but it required 60 min before its stimulus effects had completely disappeared. In contrast, nitrous oxide is not metabolized and is rapidly eliminated entirely by exhalation. In mice trained to discriminate 10 min exposure to 60% nitrous oxide, drug-appropriate responding had returned to levels near those produced by the air vehicle within only 5 min following the cessation of exposure [16]. Therefore, when inhalant discrimination training and tests are conducted outside of the inhalant exposure environment, the rapid diminution of stimulus effects necessitates very brief operant sessions, generally 2–5 min in duration. In some cases limiting the duration of the training or test session is insufficient to capture the stimulus effects of an inhalant. For example, in order to train the nitrous oxide discrimination just discussed it was necessary to employ specially designed operant conditioning/dynamic inhalant exposure chambers rather than using static exposure chambers. These dynamic chambers allowed continuous gas exposure for the duration of operant training and test sessions.

3 Inhalants as Drug Discrimination Training Stimuli

It has long been known that inhalants can serve as stimuli to control behavior [17] as well as disrupt behavior controlled by exteroceptive stimuli [18]. However it was not until the late 1980s that an inhalant was first used as the training drug in a traditional drug discrimination experiment [19]. The two initial studies one in mice and a second in rats focused specifically on training the interoceptive stimulus properties of toluene utilized administration by intraperitoneal (i.p.) injection [19, 20] rather than inhalation. Although not consistent with the manner in which it is normally abused, toluene is often administered by injection in toxicology studies [21]. Further, while route may have a major impact in some types of experiments, few studies have noted that administration route alters drug-discrimination results [22]. For instance, ethanol trained by i.p. administration in one laboratory produced comparable cross-substitution results with ethanol trained by oral gavage dosing in other laboratories [23–26]. The relative insensitivity of the drug discrimination procedure to administration route has been shown to apply to other drugs including smoke inhalation and intravenous administration of phencyclidine and cocaine [27–29] and even to self-administered versus experimenter-administered ethanol exposure [30, 31]. Among inhalants, only toluene has been compared across administration route where it has been demonstrated inhaled toluene will substitute following i.p. training in mice [19] and conversely, injected toluene cross generalizes in mice trained to discriminate inhaled toluene [32].

In 2006 our laboratory began to actively explore training inhalants as discriminative stimuli by establishing a discrimination between 10 min of exposure to 6,000 ppm inhaled toluene vapor or air [32]. The odor threshold of toluene in humans is approximately 3 ppm, which is orders of magnitude below what we believed was necessary to produce centrally mediated discriminative stimulus effects. It was a significant concern that the odor of toluene and/or its pronounced effects on the trigeminal system might serve as a preferential discriminative stimulus over its centrally mediated subjective effects [33, 34]. Indeed the exteroceptive stimulus properties of odorants have long been used as cues to control behavioral outcomes [35]. To lessen the possibility of odor cues controlling behavior, the toluene and air exposures were conducted in different experimental chambers than the discrimination training with a short temporal break between exposure and placement in the operant training chamber. Under these conditions it is required a mean of 26 sessions to train the 6,000 ppm toluene versus air discrimination, significantly faster than with an i.p. route of administration.

Several control tests were conducted to assess whether the toluene cue was due to interoceptive CNS effects, exteroceptive odor stimuli, or a compound cue composed of both CNS effects and odor. First, based on the premise that i.p. administered toluene was likely to have lower perceived odor than inhaled toluene, the ability of i.p. injected toluene to substitute for the inhaled toluene training condition was examined. When injected via the i.p. route, toluene dose-dependently and fully substituted for inhaled toluene, a result consistent with the

prior mouse study, which had demonstrated that inhaled toluene would substitute for an i.p. injected toluene training stimulus [36]. As a second control for potential olfactory stimulus effects it was demonstrated that a brief 1 min of exposure to 6,000 ppm toluene vapor did not substitute for the longer 10 min exposure training condition. Lastly, ethylbenzene, another aromatic hydrocarbon with a strong but distinctive odor, produced nearly identical levels of partial substitution for toluene regardless of whether it was administered IP or by inhalation; whereas the vapor anesthetic isoflurane produced only vehicle-appropriate responding. Interestingly, this study also suggested a reason why the prior experiments using 100 mg/kg i.p. toluene as a training stimulus required such protracted training [19, 20, 36]. Specifically, a much higher dose of 560 mg/kg i.p. toluene was required to produce full substitution in 6,000 ppm toluene vapor-trained mice. This outcome suggests that the stimulus effects of 100 mg/kg i.p. toluene are quite weak and would therefore be expected to require more extended training than the stronger 6,000 ppm inhaled toluene stimulus [37–39].

Although this experiment supported the contention that the olfactory effects of toluene were not alone sufficient to elicit toluene-appropriate responding, it did not rule out the possibility of a contributory role of odor. A subsequent experiment that again trained mice to discriminate between 10 min of 6,000 ppm toluene and air exploited toluene's inhaled pharmacokinetic properties to more thoroughly explore the role of exteroceptive versus interoceptive cue control over behavior [40]. It has been demonstrated that during extended duration exposure to toluene vapor, blood toluene levels rise rapidly for the first hour but do not entirely plateau until several hours later [41, 42]. Therefore, within the first hour of continuous exposure, toluene blood concentration can be manipulated independently from toluene odor intensity by increasing or decreasing exposure duration. Using this strategy we demonstrated across a range of exposure concentrations that 20 min of toluene exposure produced mean toluene-lever selection much greater than that produced by 10 min of exposure. Further, toluene blood concentration as quantified by gas chromatography almost perfectly predicted toluene-lever selection, a finding that has also been extended to the inhalant 1,1,1-trichloroethane [15]. Finally, toluene administered by i.p. injection had an additive effect on the stimulus effects of inhaled toluene. As a whole, these studies supported the conclusion that the stimulus effects of inhaled toluene were governed by the concentration of the drug in the bloodstream at the time of testing rather than simply by the strength of its odor. This uncoupling of stimulus effects from odor lends additional support to the conclusion that the training stimulus of inhaled toluene is primarily, if not exclusively, CNS mediated.

While toluene has been the inhalant most frequently trained as a discriminative stimulus, other inhalants have also served as training stimuli. Two different chlorinated hydrocarbon vapors 1,1,1-trichloroethane [15, 43, 44] and trichloroethylene [45] have been shown to be effective training stimuli in mice. A discrimination based on a behaviorally active, subanesthetic concentration of 6,000 ppm of the volatile anesthetic isoflurane has also been established in mice [46]. Finally, two studies in mice have reported that the discriminative stimulus effects of 60% nitrous oxide gas can be trained [16, 47]. In every case, the concentration response

functions of the training inhalants were indistinguishable from those produced by drugs trained using i.p., subcutaneous or oral gavage routes of administration. The success of these studies strongly supports the conclusion that any inhalant with sufficiently robust CNS effects can serve as an effective discriminative stimulus under the proper training conditions.

4 Pharmacological Characterization of Inhalant Discriminative Stimuli

The neurochemical and abuse-related behavioral effects of only a small number of inhalants have been explored in any detail. As previously mentioned the largest number of published reports has focused on toluene. Less attention has been given to other volatile solvents such as 1,1,1-trichloroethane and trichloroethylene. Due to their clinical importance, a fairly large literature base is available on the anesthesia-related neurochemical and behavioral effects of volatile anesthetics such as halothane, isoflurane, and sevoflurane as well as nitrous oxide gas [48]. Conversely, a number of additional inhalants with documented instances of abuse such as chlorofluorocarbons, haloalkanes, butane, propane, and nitrites have been largely neglected. As a whole the accumulated literature convincingly demonstrates that not all inhalants act upon the same receptor target or targets [for review see [6, 49].

Ligand-gated ion channels including $GABA_A$, NMDA, glycine, nicotinic acetylcholine, and $5\text{-}HT_3$ receptors appear to be particularly sensitive targets of inhalants in both in vitro and in vivo assays. The function of voltage gated ion channels are also altered by inhalants [50]. Finally, evidence exists that inhalants interact with g-protein coupled receptors including dopamine and opioid receptors. The subsequent sections of this chapter will briefly review the literature regarding the effects of inhalants on specific receptors and studies exploring whether these mechanisms are also involved in transducing the discriminative stimulus effects of individual inhalants. A summary of the studies in which probe drugs have been tested in subjects trained to discriminate inhalants is presented in Table 1.

4.1 Stimulus Effects of Inhalants: $GABA_A$ Receptors

$GABA_A$ receptors are the most abundant inhibitory ion channel receptors in the CNS and play a critical role in maintaining inhibitory tone [53]. $GABA_A$ receptors are ligand-gated chloride channel receptors composed of 5 subunits [54]. The majority of $GABA_A$ receptors have a binding sites for GABA itself as well as allosteric modulatory sites selective for benzodiazepines, barbiturates, and GABA positive neurosteroids, among others. Although many inhalants act on $GABA_A$ receptors, most [55, 56] but not all [57] studies suggest that their effects are not the

Table 1 Maximal percentage drug-lever selection of cross-test drugs in subjects trained to discriminate various inhalants from vehicle

Test drugs and mechanisms	Training drugs				
	Toluene	1,1,1-Trichloroethane	Trichloroethylene	Isoflurane	Nitrous oxide
GABA receptors					
Classical benzodiazepines	47[a] 50[b] 66[b] 72[j]	66[c] 62[d] 62[d]	48[e]	71[f]	27[h]
Zaleplon	26[b]	28[d]		74[f]	
Barbiturates	66[a] 43[b] 24[b] 34[j]	68[d]	70[e]	70[f]	10[h]
Gaboxadol		1[d]			4[h]
Muscimol				8[f]	22[h]
Neurosteroids	14[b] 8[j]				
Tiagabine		11[d]			
Valproic acid	58[b]	39[d]		85[f]	33[h]
NMDA receptors					
Uncompetitive antagonists	20[b]	13[c] 14[c] 14[d]	45[e]	44[f]	55[h] 50[h] 40[g]
CGS-19755	21[b]	25[d]	35[e]	98[f]	9[h]
L701,324	18[b]	10[d]	1[e]	24[f]	1[h]
Opioid receptors					
Morphine		4[c]			33[h]
U50,488	20[j]		22[e]	14[f]	11[h]
SNC80					10[h]
Nicotinic receptors					
Nicotine		22[c]			
Mecamylamine		1[c]			1[i]
Serotonin receptors					
8-OH-DPAT					4[h]
mCPP	19[j]		3[e]	10[f]	21[h]
MDL-72222	8[j]		2[j]		
Multiple mechanisms and other					
Ethanol	44[j]	23[d]	67[e]	52[f]	55[h] 52[g]
GHB				30[f]	
Telazol		38[d]			
Chlorpromazine	14[b]				
D-Amphetamine					1[g]
L-NAME					2[g]

[a]Reference [20]
[b]Reference [51]
[c]Reference [43]
[d]Reference [44]
[e]Reference [45]
[f]Reference [46]
[g]Reference [16]
[h]Reference [47]
[i]Reference [52]
[j]Shelton, K.L. unpublished data

results of actions at established drug binding sites. Toluene and to a lesser extent 1,1,1-trichloroethane and trichloroethylene all increase GABA-stimulated currents in $GABA_A$ receptors expressed in oocytes, but do not alter steady state $GABA_A$ receptor mediated currents. This indicates they are positive modulators rather than direct $GABA_A$ receptor agonists [58]. Toluene enhances $GABA_A$-mediated inhibitory postsynaptic currents in rat prefrontal cortex neurons [59]. Toluene, 1,1,1-trichloroethane, and trichloroethylene increase $GABA_A$ receptor mediated inhibition in hippocampal pyramidal neurons [60]. Repeated, acute exposure to toluene alters $GABA_A$ subunit expression profiles in the striatum, ventral tegmental area, and nucleus accumbens [61]. Nitrous oxide [56, 62–65], as well as volatile anesthetics, also positively modulates $GABA_A$-receptor mediated effects [66–69]. At the behavioral level, toluene attenuates convulsions induced by the $GABA_A$ receptor antagonist pentylenetetrazol [70] and demonstrates locomotor cross-sensitization with diazepam [71]. Toluene also has anxiolytic-like effects [72] similar to benzodiazepines as well as increases footshock-suppressed operant responding in mice [70] in a manner comparable to $GABA_A$-positive modulators [73].

The role of $GABA_A$ receptors in the discriminative stimulus effects of several inhalants has been examined using both cross tests of $GABA_A$ receptor ligands in animals trained to discriminate inhalants as well as cross tests of inhalants in animals trained to discriminate drugs with GABAergic mechanisms of action. In toluene-trained animals, classical non-selective benzodiazepines including midazolam, oxazepam, diazepam, and chlordiazepoxide produced partial substitution ranging from a low of 47% toluene-lever selection in rats trained to discriminate 100 mg/kg i.p. toluene from vehicle [20] up to 72% toluene-lever responding in mice trained to discriminate 2,000 ppm inhaled toluene from air [51]. Barbiturates also produce toluene-like stimulus effects under some conditions. The short-acting barbiturate methohexital elicited 66% drug-lever selection in rats trained to discriminate i.p. toluene from vehicle [20] but a much lower 24% drug-lever selection in mice trained to discriminate toluene vapor from air [51]. In mice trained to discriminate pentobarbital, toluene vapor produced greater than 85% drug-lever responding in 8 of 10 subjects [36]. In the converse training situation, pentobarbital produced greater than 80% drug-lever selection in mice trained to discriminate an extremely low dose of 100 mg/kg i.p. toluene from vehicle [19] but only 43% drug-lever selection in mice trained to discriminate a more abuse-relevant concentration of 2,000 ppm toluene vapor from air [51].

Barbiturates and benzodiazepines often cross-substitute for one another, therefore it is not surprising that both will produce some toluene-like discriminative stimulus effects. However, it does not appear that all drugs that positively modulate $GABA_A$ receptors will elicit toluene-like discriminative stimulus effects in that the GABA-positive neurosteroid allopregnanolone failed to substitute for toluene [51]. Likewise, zalaplon which preferentially binds to the benzodiazepine site in alpha 1 subunit containing $GABA_A$ receptors also failed to evoke toluene-lever responding [51]. This latter finding suggests that the benzodiazepine-like stimulus effects of toluene may not involve alpha 1 subunit containing $GABA_A$ receptors.

Classical benzodiazepines also act on $GABA_A$ receptors containing alpha 2, 3, and 5 subunits. However, selective ligands for these additional subunits have not been examined in toluene-trained mice.

The data are suggestive of a GABAergic involvement in the stimulus effects of toluene but as noted in several studies, benzodiazepines and barbiturates produced less than complete generalization making this conclusion somewhat tentative. If the same data sets are reanalyzed to take into account different sensitivities of individual subjects to the toluene-like stimulus effect of GABAergic positive modulators the data appear more convincing. For instance, at least one dose of oxazepam fully substituted for 100 mg/kg i.p. toluene in 4 of 5 rats tested [20]. Similarly, 88% of mice exhibited greater than 75% toluene-lever selection at one or more test dose of midazolam [51]. The results with barbiturates when analyzed in the same manner are less consistent. At least one dose of methohexital produced full substitution in 6 of 8 rats [20] and 4 of 5 mice [19] trained to discriminate 100 mg/kg i.p. toluene from vehicle. However in mice trained to discriminate 2,000 ppm inhaled toluene from air, one or more doses of methohexital produced full substitution in only 28% of mice and no dose of pentobarbital fully substituted in any of the subjects [51]. Unfortunately it is difficult to adequately equate these three studies given the differences in training conditions. Of these differences perhaps the most relevant was the observations that in some subjects it required in excess of 100 training sessions to establish a discrimination between 100 mg/kg i.p. toluene and vehicle whereas a mean of 65 sessions was necessary to train the discrimination between 2,000 ppm toluene vapor and air. It is therefore likely that the 100 mg/kg i.p. toluene dose was a fairly weak stimulus. Lower training doses may in some cases result in less specific discriminative stimuli, which could have been responsible for the greater degree of barbiturate lever selection in the earlier versus latter study.

While benzodiazepines substitute fairly consistently in animals trained to discriminate toluene from vehicle, in mice trained to discriminate diazepam from vehicle, toluene vapor exposure exhibited only a very low level of partial substitution [74]. This pattern of asymmetrical substitution is in many respects similar to that reported with ethanol where $GABA_A$ positive modulators as well as NMDA antagonists substitute in ethanol-trained animals more consistently than does ethanol in subjects trained to discriminate $GABA_A$ positive modulators or NMDA antagonists (for review see [75]). The findings with ethanol have been suggested to be a result of its actions on multiple receptors, which attributes ethanol with drug mixture-like properties in discrimination studies. When a drug mixture is trained as a stimulus, individual components of that mixture are sufficient to elicit mixture-appropriate responding [76–78]. However, when a single component of a drug mixture is trained as a stimulus and the mixture then tested the additional component(s) within the mixture may overshadow the common stimulus component and prevent full substitution [79]. Given that toluene, like ethanol, may interact with multiple receptors, a similar phenomenon may be taking place. However, this interpretation is speculation only as any additional components that might underlie the discriminative stimulus effect of toluene are presently unidentified.

As with toluene, classical benzodiazepines elicit partial substitution (i.e., less than 80% drug-lever appropriate responding) in mice trained to discriminate chlorinated hydrocarbons from air. Midazolam produced 66 and 62% drug-lever selection in two different studies in mice trained to discriminate 1,1,1-trichloroethane from air [43, 44]. Similarly, diazepam produced 62% drug-lever selection in 1,1,1-trichloroethane trained mice [44]. Midazolam produced a less robust 48% drug-lever selection in mice trained to discriminate trichloroethylene from air [45]. While classical benzodiazepines produced meaningful substitution for chlorinated hydrocarbons, the alpha 1 subunit preferring benzodiazepine site ligand zaleplon produced little 1,1,1-trichloroethane lever selection [44]. Interestingly, both 1,1,1-trichloroethane and trichloroethylene appear to have somewhat more robust barbiturate-like stimulus effects than does toluene. Pentobarbital elicited 68% drug-lever selection in mice trained to discriminate 1,1,1-trichloroethane from air [44] and methohexital produced 70% drug-lever responding in mice trained to discriminate trichloroethylene vapor from air [45]. In cross-substitution testing, 1,1,1-trichloroethane produced full substitution (>80%) in mice trained to discriminate 15 mg/kg pentobarbital from vehicle as well as mice trained to discriminate 20 mg/kg pentobarbital from vehicle [80] suggesting symmetry in stimulus effects between barbiturates and chlorinated hydrocarbons. As with toluene, not all positive $GABA_A$ modulators produced substitution in animals trained to discriminate chlorinated hydrocarbons as neither the extrasynaptic $GABA_A$ receptor agonist gaboxadol nor the GABA reuptake inhibitor tiagabine substituted for 1,1,1-trichloroethane [44]. The apparent differences between toluene and chlorinated hydrocarbons in their barbiturate-like stimulus effects may be due to fundamentally different receptor actions but it is equally possible they are the consequence of differential training stimulus intensities which are difficult to compare across studies.

The discriminative stimulus effects of the volatile anesthetics also appear to be partially the result of positive $GABA_A$ receptor modulation. The volatile halogenated anesthetic methoxyflurane produced full substitution in mice trained to discriminate diazepam from vehicle [74] as did halothane in mice trained to discriminate pentobarbital from vehicle [80]. The converse is also true in that both midazolam and pentobarbital produced fairly robust substitution in mice trained to discriminate isoflurane [46]. Interestingly, zaleplon produced the same level of isoflurane-lever selection as did midazolam and pentobarbital suggesting that positive modulation of alpha 1 subunit containing $GABA_A$ receptors alone is sufficient to produce isoflurane-like stimulus effects. Similar to the other inhalants previously discussed, the GABA-positive stimulus effects of isoflurane did not extend to all drugs that facilitate $GABA_A$ neurotransmission, as the direct $GABA_A$ agonist muscimol produced only vehicle-appropriate responding in isoflurane-trained mice [46]. Lastly, of those inhalants which have been examined to date, only the anesthetic gas nitrous oxide appears to be completely devoid of $GABA_A$ positive modulator-like stimulus effects [47]. Midazolam failed to substitute for 60% nitrous oxide when administered alone and when co-administered with midazolam failed to enhance the discriminative stimulus effects of nitrous oxide.

These findings suggest that nitrous oxide and midazolam do not share any discriminative stimulus properties. Likewise, gaboxadol, pentobarbital, and muscimol also only elicited vehicle-appropriate responding in nitrous oxide-trained mice.

4.2 Stimulus Effects of Inhalants: NMDA Receptors

Glutamate receptors are the primary excitatory receptors in the CNS. The NMDA receptor is one of the three subtypes of ionotropic glutamate receptors and is permeable to Ca^{2+}, Na^{+}, and K^{+}. Like the $GABA_A$ receptor, the NMDA receptor has a number of ligand binding domains [81, 82]. The channel is opened by the binding of glutamate in combination with the obligatory co-agonist glycine. The receptor can be blocked by antagonists acting through several mechanisms including those that act at the glutamate binding site, the glycine binding site, the polyamine site as well as by antagonists that bind within and block the channel itself.

There is a considerable body of literature demonstrating that a number of inhalants act as NMDA receptor antagonists in vitro and ex vivo. Benzene, xylene, ethylbenzene, and 1,1,1-trichloroethane [83] as well as isoflurane, sevoflurane, desflurane, and nitrous oxide inhibit NMDA-receptor function in recombinant receptors expressed in oocytes [65, 84–86]. Isoflurane and nitrous oxide also inhibit NMDA receptor activity in neuronal cultures and brain slices [87–91]. At the behavioral level toluene reduces the severity and lethality of NMDA-induced seizures [92] and administration of the NMDA glycine site co-agonist D-serine attenuates toluene-induced locomotor incoordination and memory impairment [93].

Drugs which attenuate NMDA receptor function by binding at the NMDA site within the channel and at the glycine co-agonist site can in many cases be differentiated from one another using drug discrimination [94–96]. The role of all three of these antagonist sites as contributors to the discriminative stimulus effects of toluene, 1,1,1-trichloroethane, trichloroethylene, isoflurane, and nitrous oxide has been fairly systematically explored. In mice trained to discriminate toluene vapor from vehicle the competitive NMDA receptor antagonist CGS-19755 produced a mean of 21% toluene-appropriate responding [51]. Likewise the uncompetitive channel blocker dizocilpine [(+)-MK-801] and the glycine-site antagonist L701,324 failed to substitute at greater than vehicle levels for toluene. These findings are consistent with previous data showing that toluene and xylene do not generalize in either C57BL/6 J or DBA/2 J mice trained to discriminate dizocilpine from vehicle [97]. However, both these studies are at odds with an experiment, which showed that 6,000 ppm toluene vapor produced a mean of 67% drug-lever selection in mice trained to discriminate 1.25 mg/kg of PCP from vehicle [74]. Dizocilpine is a highly selective NMDA receptor antagonist, whereas phencyclidine is less so. While there is no dispute that the discriminative stimulus effects of PCP are mediated by NMDA-receptor antagonism [98–100], PCP has been demonstrated to have greater downstream effects than does dizocilpine on other

neurotransmitters including dopamine and acetylcholine [101–103]. This reduced selectivity appears to have implications in drug discrimination in that several studies have shown that PCP does not always fully generalize in dizocilpine-trained animals [97, 104, 105]. The results of the study by Bowen and colleagues may therefore be detecting some additional common stimulus component between PCP and toluene that is not present with dizocilpine, the most likely of which may be amphetamine-like dopamine receptor activity [106].

Neither dizocilpine nor PCP produced any appreciable drug-lever selection in mice trained to discriminate 1,1,1-trichloroethane vapor from air [43, 44]. Likewise in mice trained to discriminate either PCP from vehicle or dizocilpine from vehicle, 1,1,1-trichloroethane produced only a low level of partial substitution [74, 97]. Dizocilpine also failed to substitute for trichloroethylene vapor in mice [45]. The NMDA receptor glycine-site antagonist L701,324 produced only vehicle-lever selection in mice trained to discriminate either 1,1,1-trichloroethane [44] or trichloroethylene from vehicle [45].

Unlike hydrocarbon solvents, the volatile anesthetic isoflurane appears to possess a NMDA antagonist-like stimulus component. The competitive NMDA antagonist CGS-19755 produced full substitution, whereas the uncompetitive antagonist dizocilpine produced partial substitution and the NMDA glycine-site antagonist L701,324 produced no substitution [46] in mice trained to discriminate isoflurane vapor. These data are in agreement with prior findings that volatile inhalants inhibit binding of ^{3}H CGS-19755 as well as ^{3}H dizocilpine [107]. The complete substitution engendered by CGS-19755 and partial substitution by dizocilpine were, however, accompanied by substantial response-rate suppressing effects such that 4 of 8 mice in each test group were excluded from the lever selection data at the doses that produced the greatest percentage isoflurane-lever selection.

NMDA receptor antagonism also appears to play a significant role in producing the discriminative stimulus effects of nitrous oxide [47]. Neither CGS-19755 nor L701,324 generalized in mice trained to discriminate 60% nitrous oxide from oxygen. However, both dizocilpine and the low-affinity NMDA channel blocker memantine partially substituted for nitrous oxide. While the substitution of dizocilpine for nitrous oxide was not complete it does appear to be selective given that a low dose of dizocilpine was also capable of significantly enhancing the discriminative stimulus effects of nitrous oxide itself.

4.3 Common Stimulus Effects of Inhalants and Ethanol

The receptor mechanisms underlying the discriminative stimulus effects of ethanol have been discussed in detail in another chapter of the present work. Briefly, $GABA_A$ positive modulators and NMDA antagonist will robustly substitute for ethanol [23, 26, 108–110]. As the discriminative stimulus of inhalants appear to be mediated by one or both of these receptors it follows that inhalants should produce ethanol-like discriminative stimulus effects. 1,1,1-trichloroethane and toluene both

produced concentration-dependent substitution in mice trained to discriminate 1 g/kg i.p. ethanol from saline [111]. Consistent with the concept of asymmetric substitution of drug mixtures in animals trained to discriminate components of that mixture, ethanol only produced partial substitution in mice trained to discriminate 1,1,1-trichloroethane, toluene, trichloroethylene, or nitrous oxide from vehicle [16, 45–47, 51]. The volatile anesthetic isoflurane, which has discriminative stimulus effects similar to both $GABA_A$ positive modulators and NMDA antagonists [46] as well as several additional vapor anesthetics, all robustly substitute in ethanol-trained mice [111, 112]. These results may be the consequence of the stimulus mixture components of volatile anesthetics and ethanol being sufficiently similar in nature that they can fully mimic one another. However, ethanol only produces partial substitution in isoflurane trained-mice [46]; therefore, their discriminative stimulus properties do not appear to be completely symmetrical as would be predicted if the relative contribution of $GABA_A$ and NMDA receptors to the stimulus effects of ethanol and volatile anesthetics were identical.

4.4 Stimulus Effects of Inhalants: Other Receptor Targets

Although $GABA_A$ and NMDA receptors are the most strongly implicated in the actions of inhalants, they are by no means the only possible ion channel receptors through which the stimulus effects of inhalants may be transduced. Toluene, perchloroethylene [113], nitrous oxide [65, 114], isoflurane, sevoflurane, and halothane [115–118] have all been shown to interact with nicotinic acetylcholine receptors. Toluene, trichloroethylene, 1,1,1-trichloroethane, and volatile anesthetics also enhance glycine receptor function [58, 119]. Lastly, isoflurane, halothane, toluene, 1,1,1-trichloroethane, and trichloroethylene all enhance $5\text{-}HT_3$ receptor function [120–122]. The role of these ion channel receptors in the stimulus effects of inhalants has received little attention. What has been established is that neither nicotine nor the uncompetitive nicotinic antagonist mecamylamine substitute for the stimulus effects of 1,1,1-trichloroethane vapor and pretreatment with either compound does not alter the 1,1,1-trichloroethane concentration-effect curve [43]. These results suggest that nicotinic acetylcholine receptors are not involved in the discriminative stimulus effects of 1,1,1-trichloroethane. This lack of nicotinic involvement may extend to nitrous oxide based on the lack of generalization of nicotine in nitrous oxide-trained mice, but this finding is tentative given the limited number of conditions examined [52]. It remains an open question as to whether nicotinic receptors may be critical to the stimulus effects of other inhalants. Finally, the $5\text{-}HT_3$ antagonist MDL-72222 does not generalize in toluene vapor-trained nor trichloroethylene vapor-trained mice (unpublished observations).

In addition to ion channel receptors, there is also some evidence supporting the hypothesis that some g-protein receptors including opioid, dopamine, and serotonin receptors are targets of inhalants. Acute exposure to toluene and 1,1,1-

trichloroethane decreased DAMGO binding to mu opioid receptors in some brain regions [123]. The kappa opioid antagonist nor-binaltorphimine (nor-BNI) and the mixed mu agonist/antagonist β-chlornaltrexamine but not the delta opioid antagonist naltrindole attenuated nitrous oxide antinociception [124, 125] in rodents. However, in humans naloxone did not alter nitrous oxide-induced changes in pain perception [126, 127].

Again, relatively little work has been done examining the extent to which opioid receptors may be involved in the stimulus effects of inhalants. It has been demonstrated that the opioid antagonist naltrexone did not attenuate the discriminate stimulus effects of 1,1,1-trichloroethane [43] nor did naltrexone alter N_2O's subjective or cognitive impairing effects in human subjects [126, 127]. The mu opioid agonist morphine produced only vehicle-appropriate responding in mice trained to discriminate 1,1,1-trichloroethane from air [43] or 60% nitrous oxide from vehicle [47]. The delta opioid agonist SNC-80 failed to substitute in nitrous oxide-trained mice [47]. Lastly, the kappa opioid agonist U50,488 failed to substitute in animals trained to discriminate trichloroethylene [45], nitrous oxide [47], isoflurane (unpublished data), or toluene (unpublished data). Interestingly, in an earlier study nitrous oxide failed to substitute in morphine-trained rats, but did substitute in rats trained to discriminate the kappa opioid agonist ethylketocyclazocine [128]. However, it was noted that naltrexone failed to block the substitution of nitrous oxide for ethylketocyclazocine, and it was suggested that the results may have not been a consequence of an interaction with opioid receptors. Some opioids such as cyclazocine share stimulus effects with the uncompetitive NMDA antagonist PCP [129] which could be the underlying mechanism for this effect. However, the stimulus effects of ethylketocyclazocine have been repeatedly demonstrated to be opioid receptor mediated [130–132], therefore the mechanism through which ethylketocyclazocine and nitrous oxide may share stimulus effects is unclear.

Repeated treatment with toluene results in reductions in dopamine D2 as well as serotonin receptor binding [133] and also produces signs of serotonin syndrome in rats including head weaving, rigidity, and Straub tail [134]. Direct infusion of toluene into the ventral tegmental area (VTA) has been shown to increase both VTA and nucleus accumbens dopamine release [135]. A high dose of i.p. toluene administered once per day for 7 consecutive days also increased dopamine and serotonin levels in some brain regions [136], as did a single 8-h period of exposure to 1,000 ppm toluene vapor [137]. In another study a shorter treatment with toluene increased locomotor activity, but did not alter extracellular dopamine levels [138]. Isoflurane also has been shown to increase extracellular dopamine release as well as inhibit dopamine transporters in synaptosomes [139], but it does not alter dopamine D2 receptor ligand binding as measured by positron emission tomography (PET) in Rhesus monkeys [140]. In contrast, a second PET study showed that isoflurane appeared to enhance dopamine transporter inhibition produced by both cocaine and the dopamine reuptake inhibitor GBR 12909 [141].

These studies suggest that inhalant effects on catecholamine receptors appear to be more common following extended or chronic exposure, which may limit their role in acute discriminative stimulus effects. A limited number of drugs altering

dopamine and 5-HT receptor function have been examined for their ability to elicit inhalant-like discriminative stimuli. Specifically, the 5-HT_{1A} agonist 5-OH-DPAT failed to produce nitrous oxide-like stimulus effects in mice [47]. The mixed 5-HT agonist m-chlorphenylpiperazine (mCPP) which has stimulus effects likely mediated by 5-HT_{2C} receptors [142–144] and has previously been shown to generalize to ethanol [145] also failed to substitute in mice trained to discriminate trichloroethylene [45], nitrous oxide [47], or isoflurane (unpublished observation) from air. A recent study failed to demonstrate that D-amphetamine had any nitrous oxide-like stimulus effects in mice [47]. However, an earlier study reported that toluene will very reliably elicit a high level of partial substitution in mice trained to discriminate D-amphetamine from vehicle [106]. These last results have yet to be replicated or extended to tests of dopaminergic agents in inhalant trained subjects, therefore the question as to whether dopamine receptor mechanisms are involved in the stimulus effects of toluene remains uncertain.

5 Cross-Substitution Studies Comparing Inhalants

The ability of drug discrimination to identify inhalants with similar stimulus effects may provide a means of rapidly classifying novel inhalants according to underlying pharmacological actions by comparing them to previously profiled reference inhalants. In order for this potential to be realized, inhalants with different pharmacological mechanisms should not cross-substitute for one another. This outcome appears likely if the pharmacological differences between two inhalants are sufficiently large. It is less certain whether cross-substitution results comparing inhalants to one another can detect more subtle differences in mechanism such as those discussed in the previous section between hydrocarbon solvents and isoflurane. This issue is not unique to inhalants but is also problematic in drug discrimination studies when comparing drugs that act upon the same receptor, but through different binding sites such as benzodiazepines and barbiturates [146].

Relatively few studies have been conducted examining the cross-substitution profiles of inhalants with one another. A summary of the results of these experiments is presented in Table 2. The most extensive inhalant cross-substitution study was conducted in mice trained to discriminate 12,000 ppm 1,1,1-trichloroethane vapor from air [15]. As previously noted, the stimulus effects of 1,1,1-trichloroethane appear to be most like those produced by positive $GABA_A$ modulators such as benzodiazepines and barbiturates [43, 44]. These $GABA_A$ positive modulator-like properties are shared by toluene [36, 51, 111] as well as trichloroethylene [45]. In 1,1,1-trichloroethane-trained mice both toluene and trichloroethylene produced complete substitution. A somewhat lower level of partial substitution was engendered by two additional aromatic hydrocarbons, ethylbenzene and o-xylene, as well as by the chlorinated hydrocarbon tetrachloroethylene. The volatile anesthetics isoflurane, desflurane, enflurane, and halothane were also tested in 1,1,1-trichloroethane-trained mice. Like 1,1,1-trichloroethane, isoflurane

Table 2 Maximal percentage drug-lever responding for volatile and gaseous compounds tested in subjects trained to discriminate different inhalants from vehicle

	Training inhalant				
Test inhalant	Toluene	1,1,1-Trichloroethane	Trichloroethylene	Isoflurane	Nitrous oxide
Aromatic hydrocarbons					
Toluene	–	100[b]	93[e]	95[d]	72[f]
Ethylbenzene	64[a]	62[b]			
O-xylene		74[b]			
Chlorinated hydrocarbons					
1,1,1-Trichloroethane		–	90[e]		44[f]
Trichloroethylene		81[b]	–	88[d]	
Perchloroethylene		70[b]	100[e]		
Volatile anesthetics					
Isoflurane	20[a]	50[b]	75[e]	–	39[f]
Desflurane		85[b]			
Enflurane		100[b]		100[d]	
Methoxyflurane			95[e]		47[f]
Halothane		100[b]		95[d]	
Other					
Nitrous oxide		15[c]		31[d]	
2-Butanol (odorant)		0[b]			3[f]

[a]Reference [32]
[b]Reference [15]
[c]Reference [44]
[d]Reference [46]
[e]Reference [45]
[f]Reference [16]

has $GABA_A$ positive modulator-like stimulus effects, but unlike 1,1,1-trichloroethane it also possesses NMDA antagonist-like stimulus properties [46]. This mixture-like stimulus profile of isoflurane is reminiscent of that produced by ethanol [75]. Ethanol produces intermediate levels of substitution in animals trained to discriminate $GABA_A$ positive modulators and this is likely due to overshadowing by the additional components of ethanol's stimulus [23, 147]. If the same concepts hold true for inhalants, it would be predicted that isoflurane should at best produce partial substitution in 1,1,1-trichloroethane-trained subjects. This was indeed the case as isoflurane resulted in a maximum of 50% drug-lever selection [15]. However, isoflurane appears to be an exception among volatile inhalants in this regard as desflurane, enflurane, and halothane all produced full substitution in 1,1,1-trichloroethane-trained mice [15, 43]. This may reflect actual differences in the pharmacology underlying the stimulus effects of volatile anesthetics, methodological factors, or inherent variability. No studies have yet been conducted using other volatile anesthetics as training stimuli to address this

question. Finally, nitrous oxide produced only vehicle-appropriate responding in 1,1,1-trichloroethane-trained mice [15], which is consistent with data indicating that nitrous oxide's stimulus is not $GABA_A$ positive modulator-like [47].

In contrast to 1,1,1-trichloroethane, the stimulus effects of nitrous oxide have an uncompetitive NMDA antagonist-like component but no $GABA_A$ positive modulator-like properties [47]. In mice trained to discriminate 60% nitrous oxide from vehicle, 1,1,1-trichloroethane, isoflurane, and methoxyflurane all produced less than 50% nitrous oxide-lever selection [16]. The poor substitution produced by 1,1,1-trichloroethane is consistent with its lack of NMDA antagonist-like stimulus effects [44]. Likewise although isoflurane has NMDA antagonist-like stimulus effects they could have been overshadowed by its GABAergic stimulus component or failed to substitute due to the stimulus effects of isoflurane being more similar to competitive than uncompetitive NMDA antagonists [46]. Interestingly toluene vapor produced a higher level of partial substitution in nitrous oxide-trained mice than any of the other inhalants which were examined [47]. This outcome is inconsistent with what would have been predicted based on the lack of NMDA antagonist-like stimulus effects of toluene [51]. One possible explanation is that both nitrous oxide and toluene possess a common but as yet unidentified stimulus component. This speculative interpretation is somewhat strengthened by the inability of any of the receptor-selective probe compounds which have been tested in toluene-trained or nitrous oxide-trained mice to fully mimic the stimulus effects of either inhalant [47, 51]. Additional studies exploring the receptors underlying the stimulus effects of both toluene and nitrous oxide will be necessary to resolve this apparent inconsistency.

Lastly, cross-substitution of several inhalants has been examined in mice trained to discriminate 6,000 ppm isoflurane vapor from air [46]. As would be predicted the related volatile anesthetic enflurane as well as halothane fully substituted for isoflurane. As previously discussed the discriminative stimulus of isoflurane appears to be composed of a $GABA_A$ positive modulator-like as well as competitive NMDA antagonist-like effects. Consistent with the notion that each of the components of a stimulus mixture is perceived as independent elements [79], both toluene and trichloroethylene which have $GABA_A$ positive modulator-like stimulus effects fully substituted for isoflurane. In contrast, nitrous oxide substitutes poorly in isoflurane-trained mice [46]. This result may be the consequence of a relatively weak NMDA antagonist-like component in isoflurane's stimulus or the dissimilarities between the stimulus effects of competitive and uncompetitive NMDA antagonists [148–150].

6 Summary and Conclusions

The mechanisms underlying the pharmacological effects of inhalants are poorly understood, especially those properties which are most important in promoting their abuse. Our lack of knowledge is exacerbated by the fact that there are dozens of

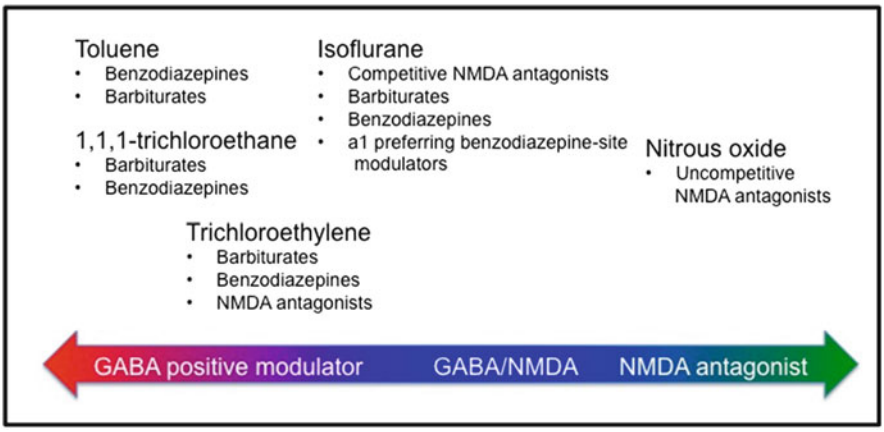

Fig. 1 Summary of greater than vehicle level cross-substitution produced by different classes of $GABA_A$ positive modulators and NMDA antagonists for five training inhalants. Classes of cross-substitution drugs below each inhalant are listed in order of greatest to least cross-substitution. Toluene and 1,1,1-trichloroethane only had $GABA_A$ positive modulator-like stimulus effects. Nitrous oxide had only NMDA antagonist-like stimulus effects. Isoflurane and trichloroethylene had mixed stimulus effects as depicted by their relative position on the X axis

different inhalants, the class is highly heterogeneous in form and structure, and many, indeed perhaps most inhalants interact with multiple receptor targets. At the present time some of the most powerful behavioral techniques (e.g., self-administration) that have proven invaluable to understanding the receptor systems involved in the abuse-related effects of other drugs have not been successfully adapted to study inhalants. Further, the toxicity of most inhalants precludes studies in humans closing off another important research strategy. Without some means of delineating the receptor systems involved in the abuse-related effects inhalants, development of pharmacological treatments to curb inhalant use and prevent relapse to inhalant abuse will continue to be seriously compromised. Drug discrimination is perhaps the most promising paradigm currently available for exploring the abuse-related effects of inhalants.

Figure 1 presents an overview of the cross-substitution results in mice trained to discriminate five different inhalants from their respective vehicles. The discriminative stimulus effects of the aromatic hydrocarbon solvent toluene, as well as the chlorinated hydrocarbons 1,1,1-trichloroethane and trichloroethylene, are mediated to a considerable extent by positive $GABA_A$ modulatory effects, similar to those produced by barbiturates and classical benzodiazepines. The lack of substitution by the alpha 1 subunit preferring nonbenzodiazepine hypnotic zaleplon also supports the argument that $GABA_A$ receptors composed of alpha 2, 3, or 5 subunits may mediate the discriminative stimulus effects of aromatic hydrocarbon solvents. There appears to be a more barbiturate-like stimulus component produced by the chlorinated hydrocarbons than by toluene, but this may be a consequence of alterations in relative selectivity produced by different training doses as opposed

to more fundamental mechanistic differences. The stimulus effects of trichloroethylene may also have a NMDA antagonist-like component although the modest level of partial substitution produced by uncompetitive NMDA antagonists makes this conclusion more tentative. In contrast, the stimulus effects of isoflurane appear to be composed of both positive $GABA_A$ modulatory actions similar to barbiturates and alpha 1 subunit selective benzodiazepines, as well as NMDA antagonist-like effects most like those produced by competitive NMDA antagonists. Finally, the discriminative stimulus effects of nitrous oxide appear to be most similar to those of uncompetitive NMDA antagonists, although incomplete substitution suggests that other as yet unidentified mechanisms are probably also involved. Finally, despite considerable data showing that inhalants alter responses mediated by other receptors the available data is not supportive of other mechanisms as mediators of inhalants acute discriminative stimuli.

Taken as a whole it appears that drug discrimination can reveal differences in the underlying neurochemical actions of inhalants that would likely be indistinguishable using other techniques. The utility of drug discrimination as a means to categorize inhalants according to abuse-related pharmacological effects is therefore encouraging. The predicative power of this technique may be constrained to some degree by the inability of drug discrimination to consistently tease apart subtle differences in specific sites of action at the same receptor. Lastly, the limited evidence available at the present time suggests that cross-substitution studies comparing novel inhalants to a panel of well-characterized archetypal reference inhalants can provide some suggestions as to the underlying neurochemical mechanisms responsible for the stimulus effects of novel inhalants. While these data may be sufficient to tentatively categorize novel inhalants according to mechanism of action, cross-substitution studies are unlikely to be as informative or as definitive as experiments in which the inhalant of interest itself serves as the training drug.

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The Discriminative Stimulus Properties of Hallucinogenic and Dissociative Anesthetic Drugs

Tomohisa Mori and Tsutomu Suzuki

Abstract The subjective effects of drugs are related to the kinds of feelings they produce, such as euphoria or dysphoria. One of the methods that can be used to study these effects is the drug discrimination procedure. Many researchers have been trying to elucidate the mechanisms that underlie the discriminative stimulus properties of abused drugs (e.g., alcohol, psychostimulants, and opioids). Over the past two decades, patterns of drug abuse have changed, so that club/recreational drugs such as phencyclidine (PCP), 3,4-methylenedioxymethamphetamine (MDMA), ketamine, and cannabinoid, which induce perceptual distortions, like hallucinations, are now more commonly abused, especially in younger generations. In particular, the abuse of designer drugs, which aim to mimic the subjective effects of psychostimulants (e.g., MDMA or amphetamines), has been problematic. However, the mechanisms of the discriminative stimulus effects of hallucinogenic and dissociative anesthetic drugs are not yet fully clear. This chapter focuses on recent findings regarding hallucinogenic and dissociative anesthetic drug-induced discriminative stimulus properties in animals.

Keywords Discriminative stimulus properties • Hallucinogens • Psychedelics • Serotonin • Sigma-1 receptor

Contents

T. Mori and T. Suzuki (✉)
Department of Toxicology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, Hoshi, Japan
e-mail: suzuki@hoshi.ac.jp

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1 Introduction

The most important determinant of a substance's abuse potential is the nature of the subjective effects that are produced by the drug's influence on the central nervous system. Alcohol, psychostimulants – like methamphetamine and cocaine – and opioids – such as morphine and heroin – produce a drug state that includes feelings referred to as euphoria. Hallucinogens and dissociative anesthetics have also been misused or abused mainly for recreational drugs. With regard to the relationship between drug-induced subjective effects and abuse potential, animal models have been developed to study the components of action of abused drugs that bear on their subjective effects in humans. One method that has considerable potential in this regard is the drug discrimination procedure, which has been used to study the mechanisms that underlie the discriminative stimulus properties of abused drugs, and the similarities among the discriminative stimulus properties of abused drugs.

Use of the club drugs 3,4-methylenedioxymethamphetamine (MDMA), including new psychoactive substances, lysergic acid diethylamide (LSD), became popular in the past few decades. Phencyclidine (PCP) and ketamine, which induce perceptual distortions (e.g., hallucinations, illusions) and disordered thinking (e.g., paranoia), are classified as dissociative anesthetic drugs. *Salvia divinorum* contains salvinorin A, which is a selective k-opioid receptor agonist and has dissociative effects, has been misused [1, 2]. On the other hand, it has been proposed that hallucinogenic effects mediated by sigma-1 receptors [3] are closely related to NMDA receptors or serotonin receptors [4, 5]. Even though these hallucinogenic drugs sometimes induce psychotomimetic effects, which are closely related to bad trips and dysphoria in humans, they have been abused for at least two decades. Interestingly, these hallucinogenic/psychedelic drugs induce both rewarding and aversive effects, depending on the details of conditioning as measured by conditioned place preference procedures in animals. While the discriminative stimulus properties of a hallucinogenic drug may be responsible for or be related to its rewarding or aversive effects, it is not yet clear exactly how the discriminative stimulus properties of hallucinogenic drugs influence for their reinforcing or aversive effects [6, 7].

Hallucinogenic drugs can be divided into distinct classes according to their chemical structures and pharmacological actions. Since the discriminative stimulus properties of a hallucinogenic drug are believed to be mediated by receptor mechanisms thought to be important for hallucinogenic effects, these drugs might substitute for the discriminative stimulus properties of other drugs (e.g., the non-hallucinogenic compound lisuride at least partially substitutes for the discriminative stimulus properties of LSD, which are mediated by the activation of

serotonergic 5-HT_{1A} and 5-HT_2 receptors) [8–10]. In most cases, each type of hallucinogenic drug exerts distinct discriminative stimulus properties. Thus, the discriminative stimulus properties of a hallucinogenic drug depend on its hallucinogenic effects and/or mechanisms of action. Several recent reports have provided new insight into the mechanisms of the discriminative stimulus properties of hallucinogenic drugs. The present chapter focuses on the mechanism(s) of the discriminative stimulus of hallucinogenic/psychotomimetic drugs. Furthermore, the possible relationship between the discriminative stimulus properties of hallucinogenic drugs and their reinforcing or aversive effects in animals was also investigated.

2 Discriminative Stimulus Effects of 5-HT-Related Compounds

MDMA and LSD (and related compounds, such as the hallucinogenic derivatives of phenethylamine and tryptamine) are known to regulate serotonergic systems to induce hallucinogenic effects. MDMA mainly releases serotonin from nerve terminals, and to a lesser extent dopamine, and, thereby, produces an enhanced mood with increased well-being or dysphoria and perceptual changes (in addition to hallucinations, illusions, and disordered thinking) in humans. Additionally, a history of MDMA use may influence the subsequent vulnerability to the use and abuse of MDMA in humans. In rodents, a large and growing body of evidence suggests that MDMA can induce hyperlocomotion and reinforcing/rewarding, aversive and discriminative stimulus properties [11].

The serotonin receptor superfamily consists of 14 subtypes that have been classified based on gene structure, amino acid sequence homology, and intracellular signaling cascades, and at least seven families of serotonin receptors (5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_6, and 5-HT_7) have been identified. Serotonin 5-HT_2 and 5-HT_{1A} receptor agonists have opposite behavioral effects; however, activation of these receptors has a synergistic action on the locomotor activity induced by MDMA [12]. The synthetic tryptamine hallucinogen *N*,*N*-dipropyltryptamine partially to fully substitutes for the discriminative stimulus properties of hallucinogens like LSD, psilocybin, and MDMA, and LSD produces MDMA-like discriminative stimulus properties in rats [13], indicating that these 5-HT-related compounds show similar discriminative stimulus properties. 5-HT_{1A} receptor agonists exert MDMA-like discriminative stimulus properties; whereas, a 5-HT_{1A} receptor antagonist partially antagonizes the discriminative stimulus properties of MDMA in rats [14]. The activation of 5-HT_{1A} receptors elicits the stimulus properties of the tryptaminergic hallucinogen 5-MeO-DMT [15], indicating that the agonist actions of 5-HT_{1A} receptors play a role in the discriminative stimulus properties of serotonin-related hallucinogenic drugs. On the other hand, it has been clearly demonstrated that the activation of 5-HT_2 receptors plays a significant role in the

discriminative stimulus properties of LSD [15]. The discriminative stimulus properties of MDMA and LSD are more potently attenuated by 5-HT_2 receptor antagonists than by 5-HT_{1A} receptor antagonists in rats [7]. The perceptual changes, emotional excitation, and adverse responses induced by MDMA are reduced by 5-HT_2 receptor antagonists in humans [16]. A more recent study showed that serotonin 5-HT_2 receptors are crucial for the reinforcing effects induced by MDMA [17]. These results indicate that the activation of 5-HT_2 receptor is an essential element of the discriminative stimulus properties and subjective effects of serotonin-related hallucinogenic drugs, which are closely related to their reinforcing and/or aversive effects, and that a 5-HT_{1A}-mediated component may have facilitatory functions [7].

It is well known that psychostimulants increase not only dopamine levels in the synaptic cleft of the terminals of the dopaminergic system, but also serotonin and noradrenaline levels. In humans, both methamphetamine and MDMA induce an increase in wakefulness and euphoria [18, 19], and it is difficult to discriminate between them based on their subjective effects in humans [20]. Thus, MDMA and other psychostimulants generally produce similar subjective effects in humans. Previous animal studies have shown that while cocaine does not substitute for the discriminative stimulus properties of MDMA, MDMA substitutes for the discriminative stimulus properties of cocaine [21]. Amphetamine partially substitutes for the discriminative stimulus properties of MDMA [22]. In contrast, MDMA does not substitute for the discriminative stimulus properties of methamphetamine [7]. In cross-substitution tests, MDMA and methylphenidate do not cross-substitute for each other in rats that have been trained to discriminate between MDMA or methylphenidate and saline [7], indicating that the discriminative stimulus properties of MDMA are distinctly different from those of other psychostimulants in rats. As mentioned above, the serotonergic system plays an important role in the discriminative stimulus properties of MDMA. However, a high dose of MDMA increases the release of dopamine, and may substitute for the discriminative stimulus properties of psychostimulants. Interestingly, recent research may provide an answer. Amphetamine substitutes for the discriminative stimulus properties of MDMA in rats that have been trained to discriminate between a high dose, but not a low dose, of MDMA and saline [23]. The discriminative stimulus properties of MDMA depend on the training doses (dopamine vs. 5-HT); lower doses of MDMA enhance serotonin, whereas higher doses of MDMA is required to enhance the dopamine release. In the case of humans, high dose of MDMA was associated with more drug-related problems [24], MDMA is frequently taken in combination with other substances to boost its effects [25]. Therefore, subjective effects of MDMA in humans are mainly mediated by the activation of serotonergic systems in the case of regular use. On the other hand, MDMA increases “negative” mood; whereas, methamphetamine enhances only “positive” mood in humans [20]. In fact, activation of dopaminergic system is partly involved in the euphoric effects of MDMA in humans [26]. In contrast, MDMA-induced perceptual changes and emotional excitation are mediated by serotonergic system [27]. Thus, MDMA and other

psychostimulants, like methamphetamine, exert some overlapping and divergent effects. Particularly, serotonin-related subjective changes may explain why MDMA and other serotonin-related drugs are used recreationally.

3 Discriminative Stimulus Effects of PCP and k-Opioid Receptor Agonist

Ketamine and PCP, which are noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists that induce a dissociative anesthetic effect, produce psychotomimetic effects, such as nightmares, hallucinations, and delusions. Noncompetitive NMDA receptor antagonists, such as PCP and MK-801, but not the noncompetitive NMDA receptor antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid, partially substituted for the discriminative stimulus properties of the barbiturate pentobarbital [28]; whereas, noncompetitive NMDA receptor antagonists, but not competitive NMDA receptor agonists, substituted for the selective k-opioid receptor agonist 2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[(1R,2R)-2-pyrrolidin-1-ylcyclohexyl]acetamide (U-50,488H) [29]. These findings suggest that the discriminative stimulus properties of competitive and noncompetitive NMDA receptor antagonists are different from each other. Further, the spectrum of behaviors induced by competitive and noncompetitive NMDA-receptor antagonists is totally different: PCP and MK-801 induce potent hyperlocomotion with ataxia, which might be related to the induction of the psychotomimetic effects of these drugs [30], whereas competitive NMDA receptor antagonists induce sedation. Since PCP, like ketamine, is not selective for NMDA receptors (i.e., PCP and ketamine can regulate the dopaminergic and serotonergic systems and sigma-1 receptor function), it is likely that several components might be involved in the cue of the discriminative stimulus properties of NMDA receptor antagonist in animals; thus, representing a "compound" or "complex" discriminative cue.

k-opioid receptors are widely distributed in regions in the brain that are closely related to rewarding effects, aversive effects, mood and cognitive functions, such as the ventral tegmental area, substantia nigra, nucleus accumbens, striatum, amygdala, locus coeruleus, hypothalamus, and dorsal raphe nucleus in human and rat brains, and are also located in the spinal cord and peripheral tissues [28], which suggests that k-opioid receptor ligands may regulate many functions in the brain. Previous studies have shown that k-opioid receptor agonists exert antinociceptive effects without producing robust reinforcing or rewarding effects. Further, k-opioid receptor agonists exert antinociceptive effects without producing robust reinforcing/rewarding effects. On the other hand, the k-opioid receptor agonist spiradoline causes sedation and dysphoria but no euphoria [31], whereas enadoline induces feelings of depersonalization in humans [32]. Furthermore, Salvinorin A also produces strong dissociative effects and memory impairment, which only partially overlap with classic hallucinogen effects [1]. Therefore, k-opioid receptor

agonists produce hallucinogenic effects and dysphoria [31, 29]. Most k-opioid receptor agonists, including salvinorin A, but not the μ-opioid receptor agonists morphine or fentanyl or the δ-opioid receptor agonist SNC80, can substitute for the discriminative stimulus properties of the prototypic k-opioid receptor agonists U50,488H and U69593 [29, 33, 34]. These previous findings indicate that the cue of the discriminative stimulus properties of k-opioid receptor agonists is not shared by the discriminative stimulus properties of other opioid receptor agonists, and closely linked to dysphoric (aversive) effects.

PCP and MK-801 substitute for the discriminative stimulus properties of U50,488H [29]. Furthermore, the discriminative stimulus properties of U50,488H, the substitution of PCP for the discriminative stimulus properties of U50,488H, and the discriminative stimulus properties of ketamine were significantly blocked by the sigma-1 receptor antagonist NE-100 ([35, 36]; for an overview of sigma-1 receptors, see next section). On the other hand, sigma-1 receptor agonists such as (+)-pentazocine and SKF10,047 completely substituted for the discriminative stimulus properties of U50,488H [35], indicating that the discriminative stimulus properties of k-opioid receptor agonists and the k-opioid receptor agonist-like discriminative stimulus properties of noncompetitive NMDA receptor antagonists are at least in part mediated by sigma-1 receptors. It should be noted here that partial substitution of fluvoxamine, which has sigma-1 receptor agonistic action [37], for the discriminative stimulus properties of MDMA was completely suppressed by NE-100. Thus, a sigma-1 receptor agonist, k-opioid receptor agonist, and noncompetitive NMDA receptor antagonist-related cue may be related to psychotomimetic-like discriminative stimulus properties.

4 Hallucination and Sigma-1 Receptors

The sigma-1 receptor agonist SKF10,047 produces hallucinogenic/psychotomimetic effects. U50,488H-induced aversive effects, which are related to its psychotomimetic potential, are completely suppressed by sigma-1 receptor antagonist [35]. Further, it was believed that the hallucinogenic effects of PCP were mediated by sigma-1 receptors. Sigma-1 receptors are specifically localized at the interface between endoplasmic reticulum (ER) and mitochondria, the so-called mitochondria-associated ER membrane (MAM) inside the ER, and regulate Ca^{2+} signaling by stabilizing 1,4,5-triphosphate (IP_3) receptors as an ER chaperone protein [38]. The activity of sigma-1 receptors could be reciprocally inhibited by an association with binding immunoglobulin protein (BiP) through the formation of a sigma-1 receptor-BiP complex. Sigma-1 receptor agonists binding to sigma-1 receptors could exhibit chaperone activity by breaking the tether of the sigma-1 receptor-BiP complex [39], and enhance the Ca^{2+} through IP_3 receptors [40]. On the other hand, a sigma-1 receptor agonist may cause a translocation of sigma-1 receptor from the MAM to the plasma membrane where the sigma-1 receptor may bind to receptors (D_1 or NMDA-receptor) or ion channels (e.g., Kv1.2 channel)

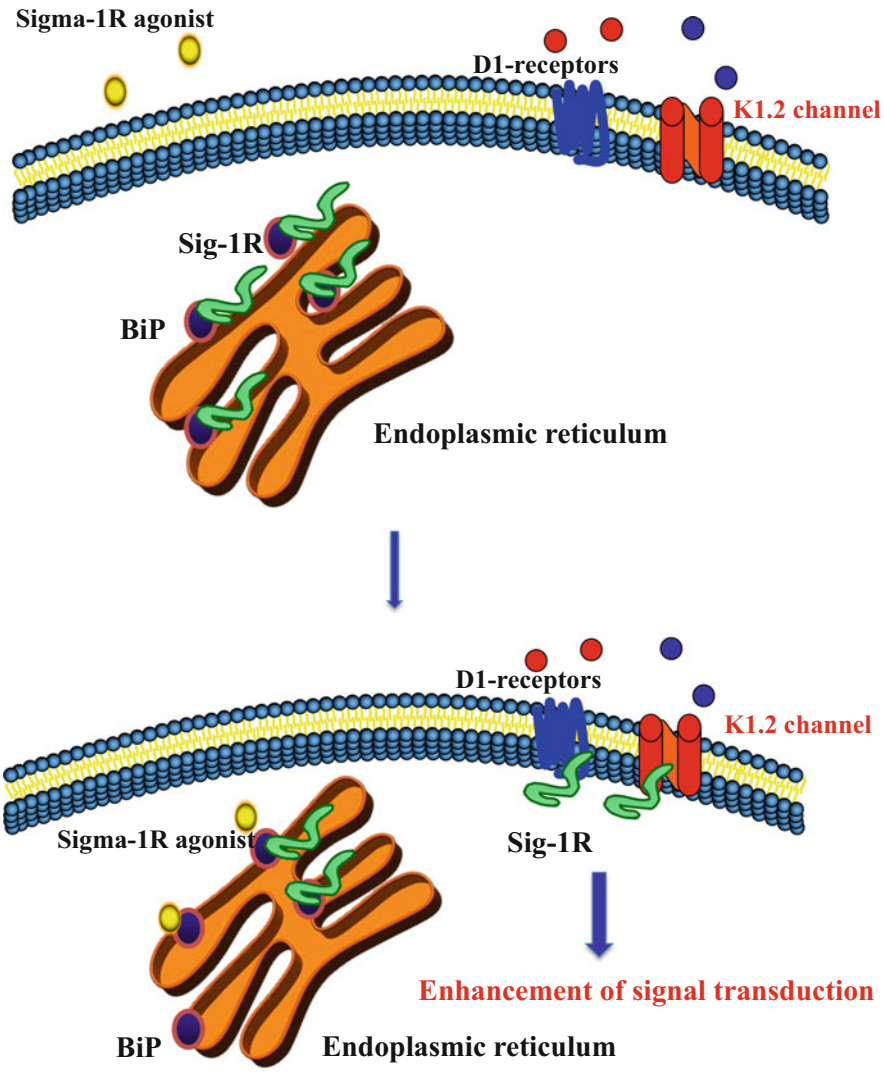

Fig. 1 Hypothetical scheme illustrating the regulation of signaling mediated by sigma-1 receptors. Sigma-1 receptors at the mitochondrion-associated endoplasmic reticulum (ER) function as ligand-activated molecular chaperones. Sig-1R agonists cause the dissociation of Sig-1Rs from another ER chaperone, binding immunoglobulin protein (BiP), allowing translocation of Sig-1Rs from ER to G-protein couples receptors and/or channels to regulate their signal transduction

that are regulating the signaling [41–43]. Recently, the endogenous hallucinogenic amine *N*,*N*-dimethyltryptamine (DMT) was shown to be an endogenous sigma-1 receptor ligand, and DMT and sigma-1 receptor agonists were shown to induce the dissociation of sigma-1 receptors from the sigma-1 receptor-BiP complex [3]. As noted above, the sigma-1 receptor antagonist NE-100 significantly attenuated the

discriminative stimulus properties of U-50,488H and the U-50,488H-like discriminative effects of PCP. However, the mechanism that underlies the involvement of sigma-1 receptors in the discriminative stimulus properties of U50,488H and the U50,488H-like discriminative stimulus properties of PCP remains unclear. One possibility is that k-opioid receptor agonists [34] as well as PCP [44] can activate extracellular signal-regulated kinase (ERK), and this activation of ERK induces the up-regulation of sigma-1 receptors [45]. Sigma-1 receptors translocated from the ER to the cellular membrane by sigma-1 receptor agonists negatively or positively regulate Src kinase, dopamine D_1 receptors, neurotropic tyrosine kinase receptor type 2 (TrkB), NMDA receptors, and $Kv_{1.2}$ channels [43, 46] (see Fig. 1). Such intracellular events might be involved in the psychotomimetic-like discriminative stimulus properties. Taken together, these results suggest that k-opioid receptor agonists and noncompetitive NMDA receptor antagonists may regulate endogenous sigma-1 receptor systems by regulating DMT, which induces a hallucinogenic effect. Therefore, the release of DMT by k-opioid receptor agonists and noncompetitive NMDA receptor antagonists should be addressed in future research.

5 Conclusion

Serotonin-related compounds and noncompetitive NMDA receptor antagonists/k-opioid receptor agonists induce hallucinations in humans and discriminative properties and reinforcing and aversive effects in animals. Previous studies have indicated that the activation of 5-HT_2 receptors plays a role in the discriminative stimulus properties of U50,488H, PCP, MDMA, and LSD in animals [15, 47]. Even though these hallucinogenic drugs induce similar behavioral phenotypes in some cases, each type of drug exerts different discriminative stimulus properties by regulating different receptors and signals. LSD and MDMA do not substitute for the discriminative stimulus properties of PCP in rats [15]. The discriminative stimulus properties of PCP were diminished by combination with LSD or MDMA in rats, presumably due to masking effects. A recent study showed that MDMA can regulate the endogenous k-opioid system mediated by the activation of 5-HT_2 receptors [48]. Therefore, it is possible that the hallucinogenic effects of U50,488H, PCP, MDMA, and LSD are mediated, at least in part, through the activation of 5-HT_2 receptors followed by sigma-1 receptors. While these drugs share some similarities in their mechanism of action, they differ with regard to the cue of their discriminative stimulus properties. On the other hand, tetrahydrocannabinol induced more robust cognitive impairment than MDMA, and their co-administration did not exacerbate the effects of either drug alone on cognitive function. However, the co-administration of tetrahydrocannabinol with MDMA increased subjective drug effects and drug strength compared with MDMA alone, which may explain the widespread use of this combination [49]. MDMA did not induce cannabinoid-like discriminative stimulus properties in rats [50]. These results suggest that cannabinoid receptor agonist has distinct discriminative

stimulus properties compared to its serotonergic-related effects. It should be noted here that humans can recognize hallucinogenic as a subjective effects induced by drugs. Nobody knows that animals could recognize whether they are having a hallucination or hallucinogenic drug-induced discriminative stimulus properties are related to hallucinogenic state, however, hallucinogenic and dissociative anesthetic drugs induce abnormal behaviors (e.g., head weaving, head-twitching, and ataxia). Furthermore, little is known about the specific regions that may part in the discriminative stimulus effects of hallucinogenic and dissociative anesthetic drugs. Such future findings may give us a better understanding of the underlying mechanisms of the discriminative stimulus effects of hallucinogenic and dissociative anesthetic drugs.

In conclusion, most hallucinogenic/psychotomimetic drugs induce distinct discriminative stimulus properties in animals, which may be related to their reinforcing or aversive effects. It is well known that most hallucinogenic drugs induce euphoria as well as dysphoria in humans depending on the situation. Thus, the discriminative stimulus properties of hallucinogens provide a reliable tool for investigating the subjective effects in humans. The discriminative stimulus properties of hallucinogenic drugs can be classified based on the underlying mechanism by which they exert their effects, such as whether they are mediated by 5-HT_2/sigma-1 (even though these receptors might be cross-linked). Based on previous results, the mechanisms of the discriminative stimulus properties of hallucinogenic drugs are related, at least partially, to their aversive effects. Interestingly, we recently interviewed 10 ex-polydrug abusers who were undergoing rehabilitation and asked them about the difference between the subjective effects of methamphetamine and hallucinogens, such as MDMA and cannabinoid. All of them stated that the subjective effects of MDMA and cannabinoid are totally different from those of methamphetamine, and there is no relapse for MDMA or cannabinoid, unlike in the case of methamphetamine. It is unclear how hallucinogenic effects may induce aversive and reinforcing effects accompanied by subjective effects/discriminative stimulus. MDMA was not a potent reinforcer in a self-administration study [6]; the ex-polydrug abusers mentioned above stated that they just enjoy the hallucination. Further research should address these points.

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Discriminative Stimulus Properties of Phytocannabinoids, Endocannabinoids, and Synthetic Cannabinoids

Jenny L. Wiley, R. Allen Owens, and Aron H. Lichtman

Abstract Psychoactive cannabinoids from the marijuana plant (phytocannabinoids), from the body (endocannabinoids), and from the research lab (synthetic cannabinoids) produce their discriminative stimulus effects by stimulation of CB_1 receptors in the brain. Early discrimination work with phytocannabinoids confirmed that Δ^9-tetrahydrocannabinol (Δ^9-THC) is the primary psychoactive constituent of the marijuana plant, with more recent work focusing on characterization of the contribution of the major endocannabinoids, anandamide and 2-arachidonoylglycerol (2-AG), to Δ^9-THC-like internal states. Collectively, these latter studies suggest that endogenous increases in both anandamide and 2-AG seem to be optimal for mimicking Δ^9-THC's discriminative stimulus effects, although suprathreshold concentrations of anandamide also appear to be Δ^9-THC-like in discrimination assays. Recently, increased abuse of synthetic cannabinoids (e.g., "fake marijuana") has spurred discrimination studies to inform regulatory authorities by predicting which of the many synthetic compounds on the illicit market are most likely to share Δ^9-THC's abuse liability. In the absence of a reliable model of cannabinoid self-administration (specifically, Δ^9-THC self-administration), cannabinoid discrimination represents the most validated and pharmacologically selective animal model of an abuse-related property of cannabinoids – i.e., marijuana's subjective effects. The influx of recent papers in which cannabinoid discrimination is highlighted attests to its continued relevance as a valuable method for scientific study of cannabinoid use and abuse.

J.L. Wiley (✉)
RTI International, 3040 Cornwallis Road, Research Triangle Park, NC 27709, USA
e-mail: jwiley@rti.org

R.A. Owens and A.H. Lichtman
Department of Pharmacology and Toxicology, Virginia Commonwealth University, Box 980613, Richmond, VA, USA

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1 Introduction

Cannabinoids are chemicals derived primarily from three sources: plants of the *Cannabis* genus (phytocannabinoids), the body (endocannabinoids), and laboratories (synthetic cannabinoids). Despite their disparate origins and different structural templates (Fig. 1), psychoactive cannabinoids bind to and activate cannabinoid CB_1 receptors, which are found in largest concentrations in the brain [1]. This mechanism underlies their ability to serve as discriminative stimuli [2]. Many cannabinoids also activate cannabinoid CB_2 receptors [3], which are primarily, but not exclusively [4, 5], located in the periphery [6]. The sections below take a critical look at preclinical cannabinoid discrimination research, in which agonists from each cannabinoid source and cannabinoid antagonists were trained as discriminative stimuli. A concluding section discusses the translational implications of this research.

2 Phytocannabinoids

In the 1960s, Mechoulam and colleagues [7] identified Δ^9-tetrahydrocannabinol (Δ^9-THC) as the primary psychoactive constituent of *Cannabis sativa*. Although Δ^9-THC is largely responsible for the subjective "high" experienced by users, the marijuana plant contains many other psychoactive and inactive cannabinoid substances, including cannabinol, cannabidiol, and Δ^8-THC [8, 9]. Major non-cannabinoid constituents of the plant include terpenoids [10]. The influence

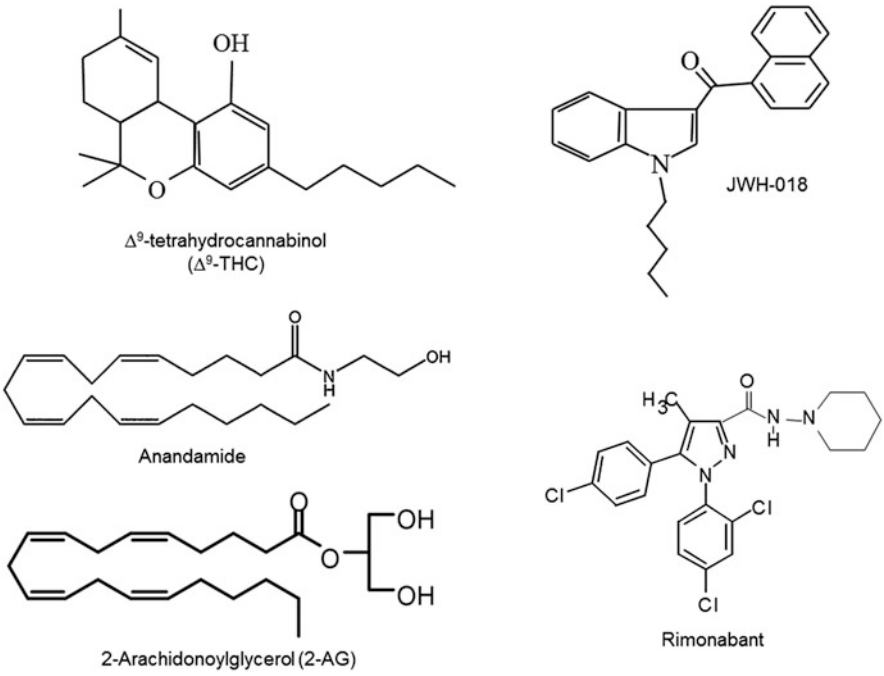

Fig. 1 Chemical structures of cannabinoids from each of the three sources: phytocannabinoids (Δ^9-THC), synthetic cannabinoids (agonist: JWH-018; antagonist: rimonabant), and endocannabinoids (anandamide and 2-AG)

of interaction(s) of these cannabinoid and noncannabinoid constituents on the pharmacological effects of Δ^9-THC has not been completely determined. Given the recent loosening of legal restrictions on use of marijuana for medicinal or recreational purposes, this area is ripe for further research.

To date, preclinical drug discrimination research with phytocannabinoids has focused almost exclusively on Δ^9-THC as a discriminative stimulus, with only a few studies reporting use of other phytocannabinoids as training drugs (e.g., [11]). Δ^9-THC's ability to serve as a discriminative stimulus has been demonstrated in rats [12], gerbils [13], pigeons [14], mice [15, 16], nonhuman primates [17], and humans [18]. Psychoactivity rests in the (−)-isomer, as (+)-Δ^9-THC does not substitute for Δ^9-THC [19]. Early studies showed that some phytocannabinoids, including Δ^8-THC, $\Delta^{9,11}$-THC, and cannabinol, substituted in Δ^9-THC-trained animals [20–22], as did Δ^9-THC's 11-hydroxy metabolites [20]. In contrast, cannabidiol, a non-psychoactive phytocannabinoid that binds to CB_1 receptors only with high micromolar affinity [23], did not generalize to Δ^9-THC [20, 24]. Later research showed that phytocannabinoid potency for producing Δ^9-THC-like discriminative stimulus effects was associated with binding affinity for CB_1 receptors in the brain [25], suggesting CB_1 receptor mediation of Δ^9-THC's discriminative stimulus effects. This hypothesis received additional support from

the finding that the prototypic CB_1 receptor antagonist rimonabant shifted the Δ^9-THC substitution dose-effect curve in Δ^9-THC-trained rats to the right [2].

Δ^9-THC's discriminative stimulus effects exhibit pharmacological selectivity for other classes of psychoactive cannabinoid agonists [17, 26, 27]. -Non-cannabinoid drugs generally fail to substitute [28]. Further, Δ^9-THC discrimination is considered a reliable animal model of marijuana intoxication [29]. As such, this model has been used to explore the physiological underpinnings of this intoxication [30] and to screen for marijuana-like abuse liability [31], as reviewed below (see Sects. 3 and 4, respectively).

3 Endocannabinoids

Discovery of CB_1 and CB_2 cannabinoid receptors in the late 1980s/early 1990s resulted in efforts to identify the endogenous substance(s) that activated these receptors, a drive that led eventually to discovery and characterization of the endocannabinoid system. In the brain, this system is one of the several lipid signaling systems and is comprised of the cannabinoid receptors, their signaling pathways, two predominant endogenous ligands, and synthetic and metabolic pathways for these endocannabinoids. To date, drug discrimination research has focused on examination of the endogenous ligands, anandamide [32] and 2-arachidonoylglycerol (2-AG) [33], and their respective primary metabolic enzymes, fatty acid amide hydrolase (FAAH) [34] and monoacylglycerol lipase (MAGL) [35].

3.1 Anandamide and Anandamide Analogs

Shortly after anandamide's discovery, investigators attempted to train rats to discriminate anandamide, an effort that failed [30, 36]. Subsequent evaluation of anandamide substitution in animals trained to discriminate Δ^9-THC or CP55,940 was inconclusive, with some studies reporting substitution [36, 37] and others reporting failure to substitute [38–40]. Similarly, systemic injection with 2-AG also failed to substitute for Δ^9-THC in a Δ^9-THC discrimination in rodents [40]. Failure of these efforts to establish a profile of the discriminative stimulus effects of endocannabinoids was attributed to their rapid metabolism [35, 41–43]. For anandamide, this hypothesis received tentative support through the finding that anandamide substituted for Δ^9-THC in Δ^9-THC-trained mice when co-administered with the nonselective amidase inhibitor phenylmethyl sulfonyl fluoride [16]. However, since reliable and selective tools to inhibit endocannabinoid metabolism were not yet available when endocannabinoids were initially discovered, cannabinoid chemists turned to synthesis of metabolically stable analogs.

These chemicals allowed examination of structure–activity relationships with the goal of determining how endocannabinoids interacted with the same (CB_1) receptor as Δ^9-THC despite notable differences in their chemical structures [44, 45]. In addition, potential physiological roles of endocannabinoids were explored through behavioral observations following administration of the analogs to living animals, including evaluation of their discriminative stimulus effects. For example, studies reported that the methylated anandamide analogs, R-(+)-methanandamide, 2-methylarachidonyl-2′-fluoroethylamide (O-875), and arachidonylcyclopropylamide, fully substituted for Δ^9-THC in Δ^9-THC-trained rhesus monkeys [37, 46]. In Δ^9-THC-trained rodents, methylated anandamide analogs with a methyl group at C-1 of the ethanolamide constituent or at C-3 of the arachidonyl group also produced the highest degree of substitution, with minimal generalization of analogs with other types of substitutions, including saturation of the arachidonyl constituent, substitution for the ethanolamide constituent or for the terminal hydroxyl [38, 39, 47]. When it occurred, substitution in rats was sometimes accompanied by decreases in overall responding [39], suggesting overlap of the stimulus effects of these compounds with Δ^9-THC. Differences were also suggested by the finding that methanandamide substituted fully in rats trained to discriminate 3 mg/kg Δ^9-THC from vehicle [48, 49], but substituted only partially or not at all in rats trained to discriminate 5.6 or 30 mg/kg Δ^9-THC [48–50]. This association of higher training doses with greater specificity in drug discrimination procedures has been noted previously for other classes of drugs [51].

To delve further into the degree to which anandamide and Δ^9-THC share cannabimimetic discriminative stimulus effects, selected anandamide analogs that substituted for Δ^9-THC in Δ^9-THC-trained animals were used as training drugs in discrimination procedures. Unlike anandamide itself, these analogs showed a reasonable degree of discriminability, and studies in rodents reported successful acquisition of discriminations for methanandamide, O-1812, and AM-1346 [50, 52–55]. In rats, Δ^9-THC also substituted for the anandamide analog used as a training drug [52, 54, 55]. Anandamide engendered greater substitution for methanandamide than it did for Δ^9-THC in rodents trained to discriminate methanandamide or Δ^9-THC from vehicle, particularly when the interval between injection and testing was short (3 min) [53] or the methanandamide training dose was high (70 mg/kg) [50]. Despite these apparent similarities between the discriminative stimulus effects of Δ^9-THC and anandamide analogs, differences also emerged. For example, Δ^9-THC did not occasion responding on the methanandamide-associated lever at a high methanandamide training dose (70 mg/kg) in mice [50]. In addition, rimonabant antagonism of Δ^9-THC's discriminative stimulus effects in rats was surmountable, whereas its antagonism of methanandamide's discriminative stimulus effects (with 10 mg/kg training dose) was not [53]. These results suggest that Δ^9-THC and rimonabant are competitive for a binding site, but that methanandamide and rimonabant interact noncompetitively. Consistent with these other differences, the discriminative stimulus effects of the 70 mg/kg training dose of methanandamide in mice were not altered by rimonabant [50], suggesting that a non-CB_1 receptor mechanism may

contribute to discrimination of high doses of methanandamide. Whether these differences are related to differences in training dose and/or species has not been determined; however, much of the recent cannabinoid discrimination research in rodents has been conducted in mice, primarily because of the facility with which this species is subject to genetic manipulation. For the most part, research with analogs of endocannabinoids in wildtype rodents has been abandoned in favor of studies with endocannabinoid metabolic enzyme inhibitors and in transgenic mice.

3.2 Endocannabinoid Discrimination in Transgenic Mice

As knowledge about the endocannabinoid system grew and the metabolic pathways for anandamide and 2-AG were delineated [35, 41, 42], transgenic mice became available and provided new opportunities to examine the potential contribution of endocannabinoid mechanisms to cannabinoid discrimination. The inability of CB_1 knockout mice to acquire a Δ^9-THC discrimination reinforced the hypothesis that the discriminative stimulus effects of psychoactive cannabinoids were mediated via activation of the CB_1 receptor [56], an idea that also received support from findings that CB_1 (but not CB_2) receptor antagonists blocked the discriminative stimulus effects of cannabinoids [57]. In mice devoid of either the catabolic enzyme FAAH or MAGL, brain levels of anandamide or 2-AG were elevated, respectively [58, 59]. $FAAH^{(-/-)}$ and $MAGL^{(-/-)}$ mice also exhibit distinct phenotypes that implicate endocannabinoid involvement in a number of physiological and behavioral processes, including pain [60, 61], seizures [62], learning and memory [63–65], and energy metabolism [66, 67].

To date, only $FAAH^{(-/-)}$ mice have been used in drug discrimination procedures. Whereas discriminability of anandamide has not been demonstrated in wildtype mice, it is readily discriminated in $FAAH^{(-/-)}$ mice in a two-lever milk-reinforced procedure [68] and in a water t-maze procedure [69]. Further, substitution of Δ^9-THC, but not the fatty acid amide oleamide, was observed in these mice. $FAAH^{(-/-)}$ mice have also been trained to discriminate Δ^9-THC in both procedures, with full cross-substitution of anandamide in these mice, but not in $FAAH^{(+/+)}$ mice tested in parallel experiments [69, 70]. While O-1812 substituted in both $FAAH^{(-/-)}$ and $FAAH^{(+/+)}$ mice, it was more potent in $FAAH^{(-/-)}$ mice [70], suggesting that the higher phenotypic brain levels of anandamide in these mice may have enhanced the cannabimimetic potency of this anandamide analog. Interestingly, co-administration of the nonspecific amidase inhibitor phenylmethyl sulfonyl fluoride, which would be predicted to increase levels of fatty acid amides such as anandamide, also enhanced the potency of Δ^9-THC and CP55,940 in Δ^9-THC discrimination in rats [71]. Rimonabant attenuation of Δ^9-THC and anandamide substitution in $FAAH^{(-/-)}$ mice trained to discriminate each drug suggests CB_1 receptor activation as a shared mechanism underlying their discriminative stimulus effects [68–70].

3.3 FAAH and MAGL Inhibitors

Recent synthesis of selective and dual FAAH and MAGL inhibitors which prevent the rapid hydrolysis of anandamide and/or 2-AG have opened up new opportunities for investigation of the psychoactive effects of endocannabinoids, particularly when combined with results from use of transgenic mice. These enzyme inhibitors produce substantial increases in brain levels of anandamide and/or 2-AG in mice [72–77]. Several selective and dual FAAH and MAGL inhibitors have been evaluated in drug discrimination alone and in combination with exogenously administered doses of anandamide or 2-AG.

Alone, selective FAAH inhibitors, URB-597 and PF-3845, and anandamide transport inhibitors, AM-404 and UCM-707, did not substitute in rodents trained to discriminate Δ^9-THC [40, 47, 78]. URB-597 also did not substitute for Δ^9-THC in Δ^9-THC-trained rhesus monkeys [79]. In combination with exogenously administered anandamide, however, URB-597 potentiated substitution of anandamide for Δ^9-THC such that full dose-dependent and rimonabant-reversible substitution was observed in rats and rhesus monkey [47, 79]. In mice, PF-3845, but not URB-597, also enhanced anandamide's efficacy in producing Δ^9-THC-like discriminative stimulus effects (maximum = 64% Δ^9-THC-lever responding), although full substitution still was not achieved [40]. Together, these results suggest that FAAH inhibition alone (with associated increases in endogenous anandamide) is not sufficient to engender Δ^9-THC-like discriminative stimulus effects without an additional influx of exogenous anandamide, suggesting that endogenous anandamide levels would not be high enough to produce Δ^9-THC-like subjective effects in humans.

As described throughout this section, most drug discrimination research with endocannabinoids has concentrated on anandamide, with less attention being given to 2-AG. The single study [40] in which 2-AG was evaluated in mice trained to discriminate Δ^9-THC reported that 2-AG did not substitute when administered alone or when co-administered with a dose of the nonselective MAGL inhibitor, N-arachidonylmaleimide [80]. A previous study had found that this dose enhanced other cannabimimetic effects of 2-AG in mice [81]. In recent years, pharmacological and genetic tools that selectively alter brain 2-AG levels have allowed emphasis to shift to examination of 2-AG's role in cannabinoid discriminative stimulus effects. While MAGL$^{(-/-)}$ mice have not yet been evaluated in a drug discrimination procedure, several MAGL inhibitors have been tested. Alone, the MAGL inhibitor JZL184 partially substituted for Δ^9-THC in wildtype mice or rats trained to discriminate Δ^9-THC from vehicle [40, 69, 70, 82], although one study reported that Δ^9-THC-trained mice responded almost solely on the vehicle-associated lever following JZL184 administration [78].

Results for other selective MAGL inhibitors have been mixed. Whereas KML29 did not substitute for Δ^9-THC in Δ^9-THC-trained wildtype mice [83], MJN110 produced full dose-dependent substitution in CP55,940-trained mice [84]. JZL184 also fully substituted for CP55,940 in these mice whereas it had only partially

substituted for Δ^9-THC in previous Δ^9-THC discrimination studies, raising the possibility that efficacy of the cannabinoid used as the training drug might affect endocannabinoid generalization profiles (CP55,940 and Δ^9-THC as full and partial CB_1 receptor agonists, respectively). Rimonabant attenuation of substitution by MJN110 and JZL184 confirmed a role for CB_1 receptor mediation in the CP55,940-like discriminative stimulus effects of these MAGL inhibitors [84].

The pattern of inconsistent substitution of MAGL inhibitors alone in cannabinoid discrimination procedures spurred investigators to examine the effects of dual inhibition of FAAH and MAGL. Dual inhibition has been achieved in one of two ways: (1) administration of a MAGL inhibitor to $FAAH^{(-/-)}$ mice and (2) administration of dual FAAH and MAGL inhibitor(s) to wildtype mice. Using the first method, studies have reported that JZL184 fully substituted for Δ^9-THC in $FAAH^{(-/-)}$ mice trained to discriminate Δ^9-THC in a food-reinforced procedure [70, 82] and partially substituted for Δ^9-THC in a water maze discrimination procedure [69]. In $FAAH^{(-/-)}$ mice trained to discriminate anandamide, a similar pattern was observed, with full substitution of the MAGL inhibitor KML29 for anandamide in a food-reinforced procedure [83] and partial substitution of JZL184 for anandamide in a water maze procedure [69]. In wildtype mice trained to discriminate Δ^9-THC, full substitution was observed with combined administration of the FAAH inhibitor PF-3845 and the MAGL inhibitor JZL184, whereas the combination of another FAAH inhibitor URB-597 and JZL184 produced responding primarily on the vehicle-associated lever [78]. This latter effect may be species-specific or related to differences in the Δ^9-THC training dose because increased responding on the Δ^9-THC-associated lever following combined administration of URB-597 and JZL184 has been reported in rats trained to discriminate Δ^9-THC from vehicle [40]. Consistent substitution for Δ^9-THC in wildtype mice trained to discriminate Δ^9-THC from vehicle has been reported for dual FAAH/MAGL inhibitors, JZL195 and SA-57 [70, 78, 82]. Substitution of both compounds was attenuated by rimonabant, suggesting CB_1 receptor mediation.

More recently, SA-57 was trained as a discriminative stimulus in wildtype mice [85]. This observation is the first reported instance of an endocannabinoid metabolic enzyme inhibitor serving as a discriminative stimulus and provides future opportunities to investigate directly the psychoactive effects of endogenous cannabinoids. In addition to demonstrating dose- and time-dependent discriminative stimulus effects, the results showed cross-substitution for CP55,940 and SA-57, with CP55,940 producing full substitution in mice trained to discriminate SA-57 and SA-57 producing full substitution in mice trained to discriminate CP55,940. SA-57's discriminative stimulus effects were blocked by rimonabant, but not by SR144528, suggesting that CB_1, but not CB_2, receptors played a role in this novel discrimination. Interestingly, SA-57 also substituted for anandamide in $FAAH^{(-/-)}$ mice. Since these mice lack FAAH, these results suggest that the increased 2-AG produced by the compound's inhibition of MAGL shares discriminative stimulus effects with anandamide. The implications of this finding for functioning of the endocannabinoid system have not yet been fully delineated.

3.4 Endocannabinoid Discrimination Summary

In summary, anandamide and 2-AG interact in a complex manner to induce cannabimimetic discriminative stimulus effects. While anandamide has been reported to substitute for Δ^9-THC, it does so only upon exogenous administration, most often with concomitant inhibition of its metabolism by FAAH. FAAH inhibitors do not induce Δ^9-THC-like discriminative stimulus effects when administered alone. In contrast, endogenous increase in 2-AG concentration via administration of an MAGL inhibitor appears more effective in promoting discriminative stimulus effects similar to those produced by Δ^9-THC and CP55,940, although variability across compounds has been noted. Further, endogenous increases in both anandamide and 2-AG seem to be optimal for mimicking Δ^9-THC's discriminative stimulus effects. Given the relatively recent availability of MAGL$^{(-/-)}$ mice and selective pharmacological tools, additional insights into the ways in which the endocannabinoid system contributes to subjective states that resemble those produced by marijuana intoxication are likely to be forthcoming as research continues in this area.

4 Synthetic Cannabinoids

Synthetic cannabinoids are a class of novel psychoactive substances that were originally developed as research chemicals to probe cannabinoid receptors and to search for compounds with potential therapeutic use. In the early 2000s, they were diverted and started to appear on drug abuse monitoring sites in products labeled "Spice" or "herbal incense" [86]. As reviewed previously [87], these compounds produce Δ^9-THC-like intoxication in humans [88, 89] and engender Δ^9-THC-like discriminative stimulus effects in rodents and nonhuman primates [27, 90]. Binding and other pharmacological properties of these compounds have been reviewed elsewhere [91, 92]. Collectively, preclinical data on these compounds have been used to support drug policy decisions in the USA, including classification of synthetic cannabinoids as schedule 1.

Previous studies have shown that CP55,940 (a bicyclic cannabinoid) and WIN55,212-2 (an aminoalkylindole) dose-dependently substituted and cross-substituted for Δ^9-THC in rodents and nonhuman primates [15, 30]. Replacement of the morpholinoethyl group of WIN55,212-2 with a pentyl chain resulted in JWH-018 (1-pentyl-3-1-naphthoylindole), the first synthetic cannabinoid identified in a Spice product. Early studies demonstrated that JWH-018 and other indole- and pyrrole-derived synthetic cannabinoids exhibit orderly structure–activity relationships in binding assays and in a battery of pharmacological tests in mice, with good correlations between CB_1 receptor binding affinities and potencies for centrally mediated cannabinoid effects [90, 93, 94]. To the extent that these compounds were tested in discrimination procedures, they also were shown to dose-dependently

substitute for psychoactive cannabinoids (Δ^9-THC, CP55,940, or methanandamide) in rats and rhesus monkeys [54, 90, 95–98], again with potencies that were consistent with their CB_1 receptor affinities. Further, substitution of JWH-018 and JWH-073 occurred following inhalation in mice trained to discriminate intraperitoneal Δ^9-THC from vehicle [99]. For some naphthoylindoles, duration of their Δ^9-THC-like discriminative stimulus effects appeared to differ from that of Δ^9-THC itself [95, 96].

JWH-018 and other structural variants of this compound comprised the first major wave of synthetic cannabinoids to be diverted for abuse. As these compounds were systematically banned, other compounds based on different structural templates quickly took their place. For example, substitution of a phenylacetyl group for the naphthoyl substituent of JWH-018 resulted in a series of phenylacetylindoles [100, 101]. Several of these compounds (JWH-203, JWH-204, JWH-205, JWH-250) have high CB_1 receptor affinity and were shown to substitute for Δ^9-THC in rodents trained to discriminate Δ^9-THC from vehicle [16, 95]; however, another compound (JWH-202) that had low CB_1 receptor affinity did not substitute [16]. XLR-11 and UR-144 are two additional compounds that have appeared on the illicit market within the last few years [102]. These tetramethylcyclopropyl ketone indoles were derived from a series described by Abbott Laboratories [103, 104] and both of these compounds produced dose-dependent substitution for Δ^9-THC in mice [31] and in rats [105]. Further, their Δ^9-THC-like discriminative stimulus effects were attenuated by co-administration of rimonabant [31], suggesting CB_1 receptor mediation.

While XLR-11 and UR-144 still occasionally appear in confiscated samples in the USA, indazole cannabinoids (e.g., AB-CHMINACA, AB-PINACA, AB-FUBINACA) have largely replaced naphthoylindoles and their derivatives as the most common compounds identified in recent samples [106–108]. As would be predicted by their high affinity for CB_1 receptors, AB-CHMINACA, AB-PINACA, and AB-FUBINACA fully substituted for Δ^9-THC in rodents trained to discriminate Δ^9-THC from vehicle [105, 109], as did the quinolinyl carboxylates, PB-22 (1-pentyl-1H-indole-3-carboxylic acid 8-quinolinyl ester) and 5F-PB-22, and the amantane-derived indole AKB-48 [105]. In contrast, the benzimidazole FUBIMINA only partially substituted for Δ^9-THC in mouse drug discrimination, which is consistent with its modest CB_1 receptor affinity [109].

As described above, synthetic cannabinoids that diverge structurally from the prototypic JWH-018 in a number of ways are Δ^9-THC-like in rodent Δ^9-THC discrimination procedures, with potencies for substitution corresponding closely to their CB_1 receptor binding affinities. In recent studies, cross-substitution between Δ^9-THC and synthetic cannabinoids has been examined. Results of two studies showed that JWH-018 served as a discriminative stimulus in rats and rhesus monkeys and that Δ^9-THC and other psychoactive cannabinoids (e.g., JWH-073) substituted for JWH-018 whereas non-cannabinoids (e.g., benzodiazepines) did not [110, 111]. JWH-018 discrimination also served as the basis for a study that examined the discriminative stimulus effects of open-ring degradants of the tetramethylcyclopropyl ketones (XLR-11, UR-144, and A834735). The formation

of these open-ring degradants are associated with repeated exposure of the parent compounds to high heat (B.F. Thomas, unpublished data), suggesting that the chemicals contained in purchased "herbal incense" products may not be identical to the chemicals the user inhales during smoking or vaping. Δ^9-THC and the open-ring degradants of the three tetramethylcyclopropyl ketones, but not a carboxy degradant of PB-22, were shown to produce full dose-dependent substitution in mice trained to discriminate JWH-018 from vehicle (J.L. Wiley and B.F. Thomas, unpublished data). Further, the open-ring degradants exhibited greater potencies and efficacies than the respective tetramethylcyclopropyl ketones from which they were derived. These findings emphasize that consideration of the actual exposure profile of the user is crucial in estimating in vivo potencies for chemicals that are inhaled after combustion or volatilization.

In an elegant study examining the relationship between efficacy and potency in a cannabinoid discrimination paradigm, Järbe et al. [112] reported successful acquisition of discriminations based upon a range of training doses of AM-5983 [(1-((1-methylpiperidin-2-yl)methyl)-1H-indol-3-yl)(naphthalen-1-yl)methanone], an indole-derived synthetic cannabinoid with high affinity and high efficacy at CB_1 receptors. Results showed that Δ^9-THC, methanandamide, WIN55,212-2, and the R- and S-isomers of AM-5983 dose-dependently substituted for racemic AM-5983. While generalization dose-effect curves for all cannabinoids showed rightward shifts as the AM-5983 training dose increased, potencies for the partial agonists Δ^9-THC and methanandamide exhibited greater enhancement than did those for the full agonists WIN55,212-2 and AM-5983. These results suggest that cannabinoid discrimination is sensitive to efficacy differences among compounds as well as differences in their potencies.

In summary, most of the discrimination research on abused synthetic cannabinoids has found that the discriminative stimulus effects of these compounds are Δ^9-THC-like in animal models, with potencies that correlate strongly with their affinities for the CB_1 receptor. Rimonabant blockade further supports CB_1 receptor mediation of the Δ^9-THC-like discriminative stimulus effects of synthetic cannabinoids [31]. To the extent tested, cross-substitution between synthetic cannabinoids and Δ^9-THC also has been demonstrated. Together, these findings are consistent with anecdotal reports that synthetic cannabinoids produce a marijuana-like intoxication [89] and continue to support the use of cannabinoid discrimination to predict abuse liability of these compounds.

5 Cannabinoid Antagonist Discrimination

Only a handful of studies have examined the discriminative stimulus effects of the CB_1 receptor antagonist/inverse agonist rimonabant. After early studies failed to establish a discrimination with rimonabant using food reinforcement [113, 114], Järbe et al. [115, 116] reported successful training of a rimonabant discrimination in rats using a taste aversion paradigm. In this paradigm, injection with the toxin

lithium chloride was systematically paired with rimonabant administration to induce discriminated aversion to a drinking solution. The toxin was not injected prior to vehicle training sessions; hence, the absence of the rimonabant cue served as a safety signal to the thirsty rats that the solution was safe to drink. Acquisition was demonstrated by consistently low levels of drinking in the presence of rimonabant and high levels of drinking in its absence (as compared to rats that received the same schedule of rimonabant and vehicle injections, but did not receive lithium chloride). Results of substitution tests in the discriminating rats showed that rimonabant and its diarylpyrazole analog AM-251 substituted whereas CB_2 receptor antagonists, SR144528 and AM630, did not [115]. Δ^9-THC also failed to substitute for rimonabant when administered alone; however, when administered in combination with rimonabant, it attenuated rimonabant's suppression of drinking, suggesting opposing actions at the CB_1 receptor [115, 116]. Hence, rimonabant appears to be discriminable, but only under modified experimental conditions.

Use of a taste aversion procedure is one way to train discrimination of a drug with low discriminability. In the case of an antagonist, a second method is to train discrimination on a baseline of chronic agonist administration. Using this method, McMahon and colleagues embarked upon a series of studies in which they successfully trained rimonabant discrimination in a shock avoidance paradigm in monkeys who were being administered ongoing daily doses of Δ^9-THC [117, 118]. Attempts to train the discrimination in monkeys that were not receiving daily doses of Δ^9-THC failed, suggesting that chronic Δ^9-THC, and the accompanying dependence, was a necessary requisite for acquisition of rimonabant discrimination. Systematic examination of the discrimination revealed that discontinuation of the daily Δ^9-THC injection produced greater responding on the rimonabant-associated lever as well as overt behaviors that were similar to those observed during cannabinoid withdrawal in other species [119]. Administration of another CB_1 receptor antagonist AM-251 also increased responding on the rimonabant-associated lever [117]. In contrast, supplemental administration of Δ^9-THC, anandamide, CP55,940 or WIN55,212-2 prior to the rimonabant training dose produced responding primarily on the vehicle-associated lever [79, 117]. Similarly, administration of non-cannabinoid drugs failed to substitute for rimonabant. Together, these results support the hypothesis that rimonabant discrimination represents a useful model with which to investigate cannabinoid dependence.

Although rimonabant does not appear to be readily trainable as a discriminative stimulus using traditional procedures in naïve animals, discrimination with a rimonabant analog O-6629 was established [120]. O-6629 is one of a series of rimonabant analogs in which various substituents have been substituted for the 3-substituent of its pyrazole core. Unlike rimonabant, however, this set of analogs produces a battery of in vivo cannabinoid effects in inbred and CB_1 knockout mice [121]. In addition, their potencies in these tests are not strongly correlated with their binding affinities for the CB_1 receptor. Results of the O-6629 discrimination study showed that O-6629 produced dose-dependent substitution for the training dose as did another 3-substituent analog O-6658 [120]. In contrast, neither rimonabant nor

Δ^9-THC substituted for O-6629. Further, O-6629 did not substitute for Δ^9-THC-trained mice and did not alter the discriminative stimulus effects of Δ^9-THC when administered in combination with the Δ^9-THC training dose. These results suggest that this set of 3-substituent rimonabant analogs represents a novel class of cannabinoids with an unknown mechanism of action.

6 Translational Aspects of Cannabinoid Discrimination

Development of a reliable model of Δ^9-THC self-administration has proven difficult [122]. To date, Δ^9-THC self-administration has not been demonstrated in rodents and has been shown in squirrel monkeys in only a single lab [123]. Consequently, cannabinoid discrimination represents the most validated and pharmacologically selective animal model of an abuse-related property of cannabinoids – i.e., marijuana's subjective effects [29]. As such, its translational implications are several. First, the increased loosening of regulations surrounding the medicinal and recreational use of phytocannabinoids in the USA and other western countries has focused attention on separation and identification of the many chemicals contained in marijuana and determination of their pharmacological effects, alone and in combination. For example, several reports have suggested that non-psychoactive constituents of marijuana (e.g., cannabidiol) may contribute to the nature of its subjective and other abuse-related effects [24, 124, 125]. In addition, therapeutic potential for various non-Δ^9-THC constituents within the plant has been proposed [126–128]. Results from cannabinoid discrimination studies have been and will continue to be helpful in characterizing interactive effects among phytocannabinoids and in determining whether a constituent proposed for medicinal use is likely to have Δ^9-THC-like effects, which may be considered aversive by inexperienced users in a medical context [129, 130]. Other potential cannabinoid medications based upon manipulation of endocannabinoid synthesis, metabolism (e.g., FAAH and MAGL inhibitors), or transport also may be screened for Δ^9-THC-like psychoactivity through the use of carefully designed Δ^9-THC discrimination studies. Synthesis of selective CB_2 agonists and peripherally restricted CB_1 receptor agonists and antagonists are additional foci of drug development efforts [131, 132], for which drug discrimination studies may predict whether lead candidates may possess unintended Δ^9-THC-like subjective effects. For cannabinoids synthesized for more nebulous purposes (e.g., "Spice," "herbal incense"), discrimination is a crucial tool in prediction of abuse liability and provision of scientific data required for classifying these compounds as schedule 1 [31, 105, 109]. Use of selective CB_1 and CB_2 antagonists in the context of discrimination of cannabinoid agonists has confirmed CB_1 receptor mediation of the discriminative stimulus effects of Δ^9-THC and psychoactive synthetic cannabinoids whereas rimonabant discrimination has been useful for examination of factors related to cannabinoid dependence. Finally, cannabinoid

discrimination provides a pharmacologically selective method for examination of underlying function of endocannabinoid system in Δ^9-THC-like intoxication.

7 Conclusions

Cannabinoid discrimination has a long history, stretching from the 1970s when the discriminative stimulus effects of Δ^9-THC initially were established to its current multi-purpose uses. Over the course of this period, drug discrimination has been used to characterize phytocannabinoids, endocannabinoids, synthetic cannabinoids, and cannabinoid antagonists. The influx of recent papers in which cannabinoid discrimination is highlighted attests to its continued relevance as a valuable method for scientific study of cannabinoid use and abuse.

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Discriminative Stimulus Properties of Opioid Ligands: Progress and Future Directions

Eduardo R. Butelman and Mary Jeanne Kreek

Abstract Opioid receptors (MOP-r, KOP-r, DOP-r, as well as NOP-r) and their endogenous neuropeptide agonist systems are involved in diverse neurobiological and behavioral functions, in health and disease. These functions include pain and analgesia, addictions, and psychiatric diseases (e.g., depression-, anxiety-like, and stress-related disorders). Drug discrimination assays have been used to characterize the behavioral pharmacology of ligands with affinity at MOP-r, KOP-r, or DOP-r (and to a lesser extent NOP-r). Therefore, drug discrimination studies with opioid ligands have an important continuing role in translational investigations of diseases that are affected by these neurobiological targets and their pharmacotherapy.

Keywords Addiction • Analgesia • Drug discrimination • Opioid • Prescription opioids

Contents

Ligands with affinity at the mu opioid receptor (MOP-r), kappa opioid receptor (KOP-r), or delta receptor (DOP-r) are among the most widely studied pharmacological classes studied in drug discrimination assays, largely preclinically, but also

E.R. Butelman (✉) and M.J. Kreek
Laboratory on the Biology of Addictive Diseases, The Rockefeller University, 1230 York Avenue, Box 171, New York, NY 10065, USA
e-mail: butelme@rockefeller.edu

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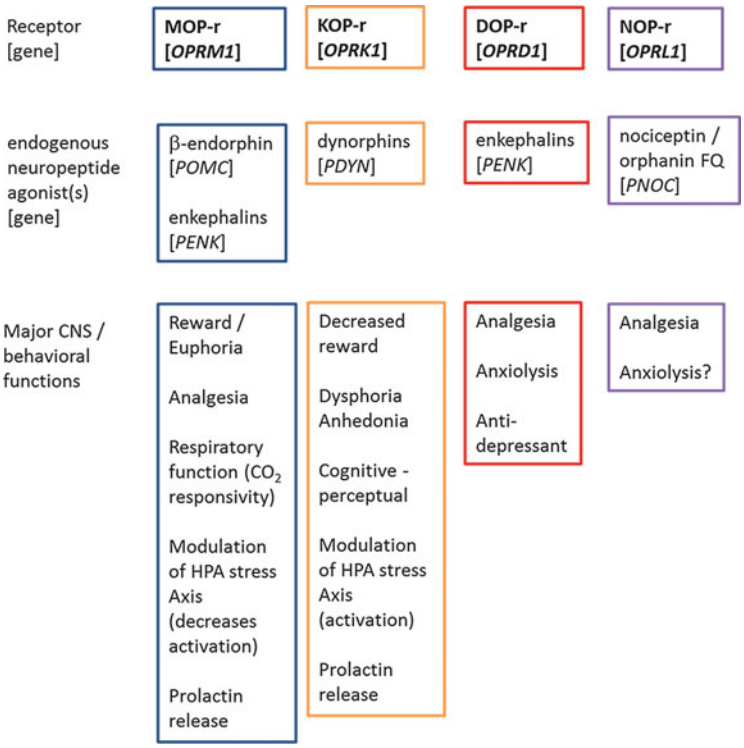

Fig. 1 Summary of opioid receptor and neuropeptide genes, and basic neurobiological and major behavioral functions. Opioid receptors (MOP-r, KOP-r, DOP-r, and NOP-r) are 7-transmembrane domain $G_{i/o}$-coupled receptors. *CNS* central nervous system, *HPA* hypothalamic-pituitary-adrenal

clinically. Ligands with affinity at nociceptin/orphanin FQ (NOP-r), another more recently discovered member of the opioid receptor family, have been studied to a lesser extent (Fig. 1). Opioid receptors and their endogenous neuropeptide agonist systems, as well as exogenous ligands, are involved in major clinical conditions, including pain and analgesia, addictions, and psychiatric diseases (e.g., depression-like, anxiety-like states, and stress-related disorders) (Fig. 1). Therefore, drug discrimination studies with opioid ligands have an important continuing role in translational investigations of diseases that are affected by these neurobiological targets and their pharmacotherapy.

Drug discrimination (as described in detail in chapters one and two in this volume) can be briefly described as a set of operant (or instrumental) techniques, by which a subject learns to emit a specific behavioral response in the presence of a particular drug stimulus (e.g., a specific dose of an opioid ligand) and a different behavioral response in the absence of the drug stimulus (e.g., when vehicle is administered). In humans, the behavioral response can be a verbal identifier (e.g., "Drug A" vs. "Drug B") or a response on a physical manipulandum. Non-operant approaches to drug discrimination have also been used, but will not be discussed extensively here. Drug discriminations are generally learnt by repeated pairings of a

particular drug stimulus with a particular reward contingency (e.g., reward by responding on a particular lever in the presence of a particular drug stimulus).

This article will focus on major fields of in vivo and behavioral opioid pharmacology studied with drug discrimination assays. The citations are not exhaustive, and we selected representative references, for brevity. Overall, several laboratories have contributed to this rich field, for methodology, pharmacological analysis/drug development, and also for the study of basic behavioral and neurobiological processes (e.g., MOP-r agonist pharmacology, tolerance, dependence, and withdrawal).

Brief Summary of Drug Discrimination Studies with Opioid Ligands

Opioid ligands (initially MOP-r agonists, such as morphine or fentanyl) figure prominently in the evolution of drug discrimination as a tool in neuroscience and pharmacology research [1]. Thus, early studies with MOP-r agonists were used to explore and develop drug discrimination techniques, and to understand the in vivo pharmacology of MOP-r and KOP-r ligands [2–5]. For example, early studies determined the pharmacological specificity of the discriminative stimulus effects of standard MOP-r agonists such as fentanyl, and their relative potency, which was positively correlated with other major in vivo effects (e.g., analgesia) [3]. Other early drug discrimination studies directly examined the then-emerging field of multiple opioid receptors [6], for example, showing that rhesus monkeys trained to discriminate standard MOP-r agonists did not generalize compounds with KOP-r agonist effects (e.g., ethylketazocine) and vice versa [4, 7]. This differentiation in discriminative stimulus effects is consistent with divergent neurobiological functions of MOP-r and KOP-r systems in CNS (see Fig. 1).

Major uses of drug discrimination assays in the opioid field include: (a) Dose- and time-dependence of discriminative stimulus effects (e.g., allowing potency and time course comparisons between ligands in the same class) [8, 9]; (b) differentiation of drug classes (e.g., MOP-r, KOP-r, or DOP-r agonists versus each other [10–13]; (c) selective antagonism, including quantitative approaches such as in vivo apparent pA_2 analysis [14–16]; and (d) characterization of novel or atypical ligands that may have an opioid-receptor mediated effect [17, 18].

Influence of the Training Dose

An important feature of drug discrimination assays in the opioid field is the influence of the magnitude of the training dose of a compound, on pharmacological specificity and identity of the interoceptive drug stimulus [19–21]. Thus, it appears that the higher the training dose of a given ligand (e.g., a MOP-r agonist), the greater the pharmacological specificity of the interoceptive drug stimulus. There are a considerable number of possibilities that could explain the aforementioned phenomenon [20, 22], including the experimental conditions under study, and differential pharmacodynamic effects at the same receptor site. For example, ligands with partial agonist MOP-r effects (e.g., nalbuphine) can be generalized by rats trained to discriminate relatively low dose of a high efficacy MOP-r agonist (e.g., fentanyl), but not to a higher training dose thereof, due to a sub-maximal "plateau" in the signaling caused by partial agonists (which can be directly characterized with in vitro signaling assays) [21, 23–25].

Table 1 Examples of exogenous opioid receptor agonists and antagonists

	MOP-r	KOP-r	DOP-r	NOP-r
Agonists	Fentanyl morphine methadone oxycodone (*and other prescription opioids*)	U50,488 U69,593 Salvinorin A	SNC80 BW373U86	Ro 64-6198 SCH 221,510
Antagonists	CTAP (peptidic) Naltrexone (*relatively low doses*) β-funaltrexamine or clocinnamox (*irreversible/functionally irreversible*)	Nor-BNI or JDtic (*very-long-lasting*) Naltrexone (*larger doses than those required to block MOP-r agonist effects*). LY645,6302 (CERC-501)	Naltrindole	J-113,397

Cross-Species and Translational Aspects

Important early contributions in opioid drug discrimination were made in rodents, pigeons, and non-human primates (see below). Drug discrimination is also one of the behavioral pharmacology methodologies that can be applied in a translational manner in humans and experimental animals, including studies with opioid ligands [26, 27]. One current obstacle to the expansion of such translational studies is the paucity (for practical, safety, and regulatory reasons) of clinically available pharmacological agents with selective KOP-r, DOP-r, or NOP-r effects. For example, most drug discrimination studies in humans exploring KOP-r function have had to employ mixed opioids (e.g., pentazocine, nalbuphine, or butorphanol) [28], which exhibit intermediate efficacy at both MOP-r and KOP-r [23, 29, 30]. By contrast, several selective MOP-r agonists can be studied in appropriately designed clinical studies (see Table 1, for selected examples). Of methodological interest, studies have also shown that specific verbal training instructions in humans can also affect the selectivity of opioid drug discrimination assays [31].

1 Mu-Opioid Receptor (MOP-r) Systems and Drug Discrimination

MOP-r systems are involved in many brain and behavioral functions, including pain, analgesia, motivation, reward and addictions, and neuroendocrine function (e.g., in the hypothalamic-pituitary-adrenal [HPA] stress axis, as well as in the prolactin release) [32]. MOP-r receptors are present in spinal and supra-spinal areas mediating the aforementioned functions. MOP-r agonists have major clinical importance as analgesics for moderate to severe pain [33], but are also drugs of abuse, and currently result in substantial mortality, mainly caused by respiratory depression [34, 35].

MOP-r agonists cause increases in activation of dopaminergic pathways, and this may be of special relevance to their euphoric and reinforcing effects, and abuse

potential [36, 37]. The prototypical MOP-r agonists morphine and fentanyl were among the earliest opioid compounds to be studied in drug discrimination assays [38–40].

Generalization Across the Discriminative Effects of MOP-r Agonists

Drug discrimination techniques have contributed considerably to our understanding of the behavioral pharmacology of these important compounds. For example, different MOP-r agonists tend to share discriminative stimulus effects, and thus are "cross-generalized" by subjects trained to discriminate a specific MOP-r agonist, across a variety of conditions and species. As an early example, when fentanyl was trained as a discriminative stimulus in rats, other structurally diverse centrally penetrating MOP-r agonists (e.g., methadone and morphine) were generalized [38]. In a different example, rhesus monkeys trained to discriminate heroin generalized to its active metabolites including morphine and morphine-6-glucuronide, and structurally diverse MOP-r agonists, such as fentanyl and methadone [41]. This profile has allowed the use of drug discrimination assays to investigate abuse potential of novel MOP-r compounds or formulations. Thus, it can be postulated that novel compounds that do not generalize to abused compounds such as classic MOP-r agonists may present lesser abuse liability.

Pharmacological Specificity of MOP-r Agonist Discriminative Stimulus

One of the strengths of drug discrimination as an in vivo assay is that can exhibit prominent pharmacological specificity, in that compounds that have similar pharmacodynamic effects tend to produce similar discriminative stimuli (see above). Thus, if an MOP-r agonist is the training compound in a drug discrimination assay, other MOP-r agonists are generalized, whereas compounds from other pharmacological classes (such as KOP-r or DOP-r agonists, or compounds acting at other systems) are typically not generalized [14, 41–43]. This feature is especially useful for the study of novel compounds at doses below those that may produce overt behavioral effects, or for classes of compounds that may share some overt behavioral effects (such as antinociception or locomotor effects), but can be differentiated by their discriminative effects in animals and potentially in humans. One major clinically relevant example of such pharmacological specificity is the finding that mixed opioid ligands (such as pentazocine) can be differentiated from a selective MOP-r agonist (e.g., hydromorphone) in a three-way discrimination, that is, pentazocine vs hydromorphone vs. saline. A likely reason for such a profile is that mixed opioids such as pentazocine also have KOP-r mediated effects, in addition to intermediate efficacy at MOP-r receptors [26, 44]. The discriminative stimulus effects of such "mixed opioids" (including widely used compounds such as buprenorphine) vary across species. This is possibly due to differential receptor selectivity or differential signaling efficacy across species [30, 45–47].

Differential Pharmacodynamic Efficacy of MOP-r Ligands as Detected in Drug Discrimination Studies (High Efficacy Agonists Versus Partial Agonists)

Under specific conditions, drug discrimination studies can also be used to differentiate pharmacodynamic (or signaling) efficacy of MOP-r ligands. As mentioned

above, when a relatively high dose of the high efficacy MOP-r agonist fentanyl was trained as discriminative stimulus in rats, other high efficacy agonists such as methadone could be generalized; whereas, compounds with MOP-r partial agonist effects (e.g., nalbuphine) were not fully generalized, presumably because they were unable to produce a neurobiological signal of the required intensity [21].

A second type of approach that has been used to detect differences in pharmacodynamic efficacy in drug discrimination assays includes the use of ligands that cause irreversible or functionally irreversible MOP-r antagonism, such as β-funaltrexamine or clocinnamox (see Table 1), which cause a decrease in MOP-r B_{max} [16, 48, 49]. As in other in vivo assays (such as antinociception), discriminative effects of partial agonists such as morphine were more sensitive to such a decrease in available MOP-r populations, than higher efficacy agonists such as fentanyl [16, 50, 51]. See also below for further discussion of tolerance to discriminative effects of opioids.

Overall, these differential discriminative patterns of partial MOP-r agonists versus higher efficacy agonists can potentially inform preclinical, clinical, and regulatory investigators to the relative profile and abuse potential of novel compounds, including compounds with effects partially mediated through non-opioid receptor mechanisms [18, 52–55].

Drug Discrimination Studies of MOP-r Agonist Tolerance

Repeated exposure to MOP-r agonists is known to result in tolerance, that is, a decrease in the observed effect of a given dose (often quantified as a rightward shift in agonist dose-effect curves). Tolerance can be observed clinically both in analgesia and drug addiction settings, and is of relevance to acute MOP-r toxicity (i.e., respiratory depression) in persons with different amounts of MOP-r exposure [33, 56]. Tolerance per se, in the context of appropriate medical control of pain, is not indicative of abuse or addiction. Neurobiological mechanisms of tolerance may differ across in vitro and in vivo endpoints, and are not fully understood, despite intensive study [57]. Tolerance has thus been detected after repeated MOP-r agonist exposure in experimental animals, in some, but not all drug discrimination studies [58–61]. For example, in rats trained to discriminate morphine from vehicle, dose-effect curves for morphine and other MOP-r agonists were shifted to the right (i.e., a decrease in potency observed due to tolerance) due to chronic morphine exposure (14–18 days). In this study [61], training was suspended during the chronic morphine exposure period, to minimize the risk of "re-training" to functionally decreasing doses of the training drug [61]. Differential methodological factors in training and testing (including the aforementioned suspension of training during chronic MOP-r agonist exposure) may underlie these apparent differences.

Drug Discrimination Studies of MOP-r Agonist Dependence and Withdrawal

Other neurobiological and behavioral hallmarks of chronic MOP-r agonist administration in humans and experimental animals are dependence and withdrawal. For example, repeated administration of MOP-r agonists (and of several other types of compounds, not necessarily all abused) results in neurobiological and behavioral adaptations that can be discerned upon drug discontinuation as a "withdrawal syndrome." The classic MOP-r agonist withdrawal syndrome is aversive (with a variety

of subjective effects, including anxiogenesis) and includes autonomic/sympathetic over-activation, piloerection, tremors, diarrhea, and hypothalamic-pituitary-adrenal (HPA) hormonal axis activation [56, 62–64]. Several neurobiological and molecular mechanisms of MOP-r agonist dependence and withdrawal have been investigated [65–67]. Avoidance and escape from MOP-r withdrawal can be studied as processes underlying negative reinforcement in experimental animals [68, 69]. Similarly to tolerance above, the presence of dependence or withdrawal in the context of appropriate medical care for pain is not alone indicative of abuse or addiction.

Drug discrimination studies have also been used to characterize internal stimuli of withdrawal, in that animals that receive chronic MOP-r agonists (e.g., morphine) can be trained to respond differentially when drug is acutely discontinued, or when there is precipitated short-term withdrawal caused by a relatively small dose of an opioid antagonist (e.g., naloxone or naltrexone) [70]. For example, in rhesus monkeys chronically treated with morphine, a low dose of the opioid antagonist naltrexone can be trained as a discriminative stimulus, and short-term morphine discontinuation can be generalized to the naltrexone stimulus [70, 71]. Like other opioid discriminative stimuli, this endpoint can be sensitive and repeatable, and can be examined under conditions that do not cause robust overt withdrawal signs. Some drug discrimination studies also have investigated the phenomenon of "acute withdrawal," in which a single relatively large dose of MOP-r agonist is rapidly followed by treatment with an antagonist such as naloxone or naltrexone. This acute withdrawal may share some behavioral and neurobiological similarities to classic withdrawal mechanisms observed after chronic MOP-r agonist exposure (mentioned above) [72].

Overall, MOP-r antagonists alone (in the absence of MOP-r agonist exposure, see above) have low potency or effectiveness as discriminative stimuli. This may be an indication that in unperturbed subjects, major MOP-r systems have relatively low basal "tone" (i.e., limited endogenous agonist occupancy or receptor signaling). One exception may be the hypothalamic-pituitary-adrenal (HPA) axis, in that compounds such as naloxone and naltrexone can acutely cause increases in levels of stress hormones ACTH and cortisol, in human and non-human primates, in the absence of chronic MOP-r agonist exposure [73, 74].

Of interest, compounds used clinically to decrease certain withdrawal signs (such as the adrenergic α_2-agonist clonidine) do not robustly or dose-dependently block the postulated "withdrawal" discriminative stimulus, even though they partially block some subjective, overt and autonomic effects, in humans and non-human primates [70, 75, 76]. This is an illustration of drug discrimination as a practical tool to examine clinically important interoceptive experiences of withdrawal, which may be dissociated from overt signs, and may be of relevance to processes of continued addiction and relapse.

Relationship of Discriminative Effects of MOP-r Agonists to Other Behavioral, Physiological and Neurobiological Effects, and Abuse Potential

There is typically a positive correlation between the potency (e.g., ED_{50} values) of centrally penetrating MOP-r agonists in causing discriminative stimuli and in causing clinically relevant in vivo effects, including antinociception/analgesia, respiratory

depression, and reward-related effects [77, 78]. Generally, operant drug discrimination tends to occur at smaller doses than some of the aforementioned endpoints (when studied within a species and route of administration). Thus, discriminative stimulus effects may be a useful predictive biomarker for these other effects of relevance to preclinical and clinical evaluations, including for abuse potential.

Generalization of novel compounds to the discriminative effects of known drugs of abuse (e.g., MOP-r agonists) is indicative of abuse potential, of relevance during the drug development process, and to regulatory evaluation [55, 79, 80]. In a related manner, compounds with opioid receptor components of action such as the analgesic tramadol can be evaluated for cross-generalization to standard MOP-r agonists (e.g., the prescription opioid hydromorphone) [18].

2 Kappa Opioid Receptor (KOP-r) Systems and Drug Discrimination

KOP-r receptors and their endogenous high efficacy agonist ligands (the dynorphins) are also widely distributed in areas in the CNS mediating motivated behaviors and euphoria/dysphoria (e.g., caudate–putamen and nucleus accumbens), learning, memory, and emotional processing (e.g., hippocampus and amygdala), neuroendocrine function (e.g., hypothalamus), as well as several cortical areas [81–83]. Exogenous KOP-r agonists tend to cause aversion and dysphoria, and also sedation and psychotomimetic or hallucinogenic effects, as investigated in preclinical and clinical studies [12, 84–86]. Of interest, KOP-r agonists tend to decrease synaptic dopamine overflow, an effect opposite to that of MOP-r agonists and other abused compounds, including cocaine and ethanol [36, 37, 87]. Plasticity in KOP-r/dynorphin systems (typically upregulation) has been detected experimentally after exposure to different drugs of abuse or diverse stresses; this appears to be a mechanism underlying escalation of drug taking, and relapse-like, depressant-like, or anxiety-like behaviors [88–92].

Selective KOP-r agonists or antagonists have been examined in several clinical studies [93–95], but have not been examined in formal drug discrimination studies in humans, to our knowledge. Of interest, salvinorin A, a diterpene derived from the plant *Salvia divinorum*, is a selective high efficacy KOP-r agonist and there has been a recent expansion in its non-medical use (see below) [96]. As alluded to above, clinically used compounds such as buprenorphine, nalbuphine, and butorphanol have considerable affinity at both MOP-r and KOP-r, and have intermediate signaling efficacy, which can vary according to experimental situation [29, 30].

Specificity of Discriminative Effects of KOP-r Agonists

Selective exogenous KOP-r agonists produce discriminative stimulus effects that are differentiated from those of MOP-r or DOP-r agonists, as studied in different non-human species [12, 14, 97]. Selective KOP-r agonists, be they synthetic or plant-derived (such as salvinorin A, from the plant *Salvia divinorum*, the focus of considerable non-medical use) produce dissociative, psychotomimetic, or

hallucinogenic effects in humans [84, 85]. However, KOP-r agonist-induced discriminative effects are distinct from those of pharmacologically unrelated compounds that produce dissociative or hallucinogenic effects in humans, including 5HT2A agonists such as LSD or psilocybin, or NMDA antagonists such as ketamine [98, 99]. Of translational relevance, human subjects who received salvinorin A have also reported that the interoceptive or experiential/subjective effects of this KOP-r ligand may differ from those of classic hallucinogens, for example, by causing more intense dissociative and somatic effects [100, 101]. This further illustrates the degree of in vivo pharmacological selectivity that can be afforded by drug discrimination assays, since such behavioral or experiential/subjective effects (dysphoria, dissociation, hallucinations, or psychotomimetic effects) may be ultimately produced by ligands acting directly on different receptor systems and neuronal pathways [102]. This suggests a continuing potential contribution of drug discrimination studies to more mechanism-based analysis of such experiential/subjective effects [103, 104]. The relative potency of centrally penetrating KOP-r agonists in drug discrimination assays is also positively correlated with their potency in several other behavioral assays, including clinically undesirable effects such as sedation, and also with the translational neuroendocrine biomarker assay of prolactin levels [12, 93, 105].

3 Delta Opioid Receptor (DOP-r) Systems and Drug Discrimination

Peptidic and non-peptidic DOP-r agonists produce characteristic discriminative effects that can be differentiated from those of MOP-r and KOP-r agonists, typically by a lack of cross-generalization (also, peptidic DOP-r agonists have been typically administered by the i.c.v route, to bypass their exclusion by the blood–brain barrier) [10, 14, 106]. As an example, rhesus monkeys trained to discriminate the DOP-r agonist SNC80 exhibited at most nondose-dependent partial generalization when administered MOP-r or KOP-r agonists (morphine and U50,488 respectively) [10]. Likewise, selective antagonism studies also confirm the pharmacological specificity of these stimuli. For example, the DOP-r selective antagonist naltrindole can potently block the discriminative effects of the synthetic DOP-r agonists SNC80 or BW373U86 [10, 14]. These studies further illustrate the pharmacological specificity of drug discrimination assays, and their use in the characterization of novel ligands, including those that may produce subtle unconditioned behavioral effects.

Nociceptin/Orphanin Systems and Drug Discrimination

The NOP-r receptor system (and its endogenous agonist, nociception/orphanin FQ) have some genetic, functional and neuroanatomical homology to MOP-r, KOP-r, and DOP-r systems [107]. Certain clinically used ligands, such as buprenorphine, do have NOP-r mediated effects, although it is unknown to what extent these are important to their clinical profile [108]. NOP-r ligands have been investigated for different

pharmacotherapeutic indications, especially analgesia [109–111]. Based on the limited number of available studies in experimental animals [112, 113], NOP-r agonists also produce characteristic discriminative stimulus effects, distinct from those of MOP-r, KOP-r, or DOP-r agonists. Thus, in rats trained to discriminate the NOP-r agonist Ro 64-6198, morphine produced a maximum of 40% drug-appropriate responding, and KOP-r and DOP-r agonists each produced less than 25% drug-appropriate responding (mean values) [112]. In the same study, Ro 64-6198 only produced $\leq$25% morphine appropriate responding in a separate group of rats. This suggests that NOP-r agonists do not share interoceptive effects of standard agonists at MOP-r, KOP-r, or DOP-r.

Please see Table 1 for a summary of major agonists and antagonists that can be potentially useful for drug discrimination studies examining the above opioid receptor systems.

4 Current Trends and Potential Future Directions

As previously noted, drug discrimination assays with opioid ligands continue to have an important role in behavioral pharmacology, including studies of abuse potential in novel drugs. Thus, novel opioid ligands with discriminative stimulus effects different from those of MOP-r agonists such as heroin or abused prescription opioids could be considered to have decreased likelihood of abuse potential.

"Biased" Ligands

The pharmacological specificity of drug discrimination assays can also be used to investigate timely mechanistic questions in the larger opioid neurobiology field. For example, it is unknown to date whether opioid ligands acting at the same receptor, but with different downstream signaling "bias" (e.g., at G-protein, adenylyl cyclase, β-arrestin, and other downstream pathways) would have differential discriminative stimuli [114, 115]. For example, it would be of interest to determine whether MOP-r ligands with differential "bias" also have differential discriminative effects, as this may be relevant to their profile of desirable and undesirable effects in the clinic (e.g., analgesia vs. abuse potential).

Drug Discrimination as a Behavioral "Readout" in Studies of Opioid Neurobiology, and as Dimensional Variables

Drug discrimination assays, due to their relative robustness, repeatability, and quantitative nature, are suitable for relatively low "n" studies. Such "behavioral readouts" can be useful in parallel with other techniques, including PET or fMRI neuroimaging, to provide a measure of the interoceptive qualities associated with a particular neuroimaging signal (e.g., receptor occupancy by a particular ligand, acting at MOP-r, KOP-r, or DOP-r). The pharmacological specificity of discriminative stimuli (mentioned above) would also be an asset in such biomarker studies. A relatively small number of studies have investigated the neuroanatomical site (s) of opioid-receptor mediated discriminative effects [116–118], and this may be potentially investigated further with neuroimaging approaches.

The potential of discriminative effects of opioids to be used as "dimensional" variables for the study of mental health, pain/analgesia, and specific addictions can also be considered [119]. For example, drug discrimination studies can be designed comparatively for humans and experimental animals, allowing translational mechanistic studies. In addition, drug discrimination dose-effect curves can be examined as dimensional markers for underlying neurobiological and pharmacodynamic mechanisms.

Sex Differences

Sex differences in discriminative effects of opioids have been studied primarily in rodent models [120, 121]. Some of the sex differences reported include a greater potency of the discriminative effects of the MOP-r agonist morphine in females vs. male rats, whereas the converse was observed for the discriminative effects of the KOP-r agonist U69,593 [120, 122]. Further studies on sex differences in discriminative effects of specific opioid ligands are of importance, for both basic and clinical science [123]. For example, a mechanistic investigation of the aforementioned sex differences in MOP-r induced discriminative effects may be translationally relevant to differential profiles of abuse of MOP-r agonists in women and men [124, 125].

Genetics, Epigenetics, and Clinical Status in Drug Discrimination Studies

Opioid receptor systems (and their endogenous ligands) exhibit clinically relevant genetic polymorphisms (e.g., at *OPRM1*, *OPRK1*, and *OPRD1*, as well as *POMC*, *PENK1*, and *PDYN*), and are also affected by epigenetics and environmental history [126–130]. Opioid receptor and neuropeptide systems are altered (for example, at the mRNA or protein level), by exposure to stress or to drugs of abuse, of relevance to diverse mental health conditions (see reviews) [89, 131]. Therefore, discriminative stimulus effects of opioid ligands could be hypothesized to differ based on such genetic, epigenetic, and stress/environmental factors, and this could be studied in appropriate animal models, including transgenic constructs, as well as in specific clinical populations. Overall, drug discrimination has the potential to remain a powerful methodology for modern neurobiological, neuropharmacological, and translational studies.

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Translational Value of Drug Discrimination with Typical and Atypical Antipsychotic Drugs

Joseph H. Porter, Kevin A. Webster, and Adam J. Prus

Abstract This chapter focuses on the *translational value* of drug discrimination as a preclinical assay for drug development. In particular, the importance of two factors, i.e., training dose and species, for drug discrimination studies with the atypical antipsychotic clozapine is examined. Serotonin receptors appear to be an important pharmacological mechanism mediating clozapine's discriminative cue in both rats and mice, although differences are clearly evident as antagonism of cholinergic muscarinic receptors is important in rats at a higher training dose (5.0 mg/kg) of clozapine, but not at a lower training dose (1.25 mg/kg). Antagonism of α_1 adrenoceptors is a sufficient mechanism in C57BL/6 and 129S2 mice to mimic clozapine's cue, but not in DBA/2 and B6129S mice, and only produces partial substitution in low-dose clozapine discrimination in rats. Dopamine antagonism produces partial substitution for clozapine in DBA/2, 129S2, and B6129S mice, but not in C57BL/6 mice, and partial substitution is seen with D_4 antagonism in low-dose clozapine drug discrimination in rats. Thus, it is evident that clozapine has a complex mixture of receptor contributions towards its discriminative cue based on the data from the four mouse strains that have been tested that is similar to the results from rat studies. A further examination of antipsychotic stimulus properties in humans, particularly in patients with schizophrenia, would go far in evaluating the translational value of the drug discrimination paradigm for antipsychotic drugs.

Keywords Antipsychotic drugs • Clozapine • Drug discrimination • Mouse strains • Training dose • Translational models

J.H. Porter (✉) and K.A. Webster
Department of Psychology, Virginia Commonwealth University, Richmond, VA 23284, USA
e-mail: jporter@vcu.edu

A.J. Prus
Department of Psychology, Northern Michigan University, Marquette, MI, USA

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Contents

This chapter, located within the volume *The Behavioural Neuroscience of Drug Discrimination* as part of the *Current Topics in Behavioral Neurosciences* series, reviews the major findings from the experimental literature to describe the current state of knowledge on the discriminative stimulus properties of antipsychotic drugs. As described below, much of that research has focused on clozapine (Clozaril®), which has remained as the "gold standard" among the atypical (second-generation) antipsychotics. One obvious goal of preclinical research is to develop assays that can be used for the development of new pharmacotherapeutic drugs that are more efficacious and have fewer side effects. While drug discrimination is not a model of human disorders like depression or schizophrenia, it does quantitatively measure the subjective effects of drugs (from different behavioral/therapeutic classifications) and their subjective (i.e., interoceptive) effects produced by antipsychotic drugs as well as by many others. Such subjective effects are primarily mediated by a drug's pharmacological action (typically, blockade or activation) at receptor sites. Thus, in addition to predicting the therapeutic efficacy of novel compounds, drug discrimination provides a universally straightforward and sensitive measure of how drug binding at the receptor level can influence conditioned behavioral events in vivo.

1 Treatments for Schizophrenia

Until 1952 and the discovery of the first antipsychotic drug chlorpromazine in France, there were no effective treatments for the symptoms of psychosis or other mental illnesses. Chlorpromazine was initially marketed as an antipsychotic in France in November 1952 as Largactil® ("large in action") and later in the USA in March 1954 as Thorazine® [1]. Chlorpromazine's ability to effectively treat psychotic symptoms marked the birth of psychopharmacology and provided a concrete link to organic roots of mental illnesses – e.g., the dopamine hypothesis of schizophrenia first proposed by Jacques M. van Rossum ([2]; for reviews see Baumeister and Francis [3] and Snyder et al. [4]). Perhaps the biggest impact of antipsychotic drug treatments was their ability to allow hospitalized schizophrenics to live relatively normal lives

outside of mental hospitals and asylums. In fact, the therapeutic use of drugs to treat the symptoms of schizophrenia, depression, and anxiety initiated a significant reduction in institutionalized patients that began in the 1950s with a historic high of 559,000 patients in 1955 and continued over the next 30 years to 107,000 in 1988 [5]. One of the goals of psychopharmacology was to better understand the behavioral effects and neuropharmacological properties of these new therapeutic drugs that were rapidly being placed into clinical use throughout the 1950s and 1960s. It became apparent fairly early in clinical use that these first-generation ("typical") antipsychotic drugs (neuroleptics) produced extrapyramidal motor side effects (EPS) that resembled Parkinsonian symptoms, which appear to be due to antagonism of dopamine receptors. In fact, for many years clinicians believed that EPS and the therapeutic efficacy of the first-generation antipsychotics were explicatively linked. It was not until the atypical antipsychotic clozapine was found to be as (and perhaps more) effective for the treatment of schizophrenia that this belief was dispelled [6, 7]. One major difference in clozapine was that it differed from typical antipsychotics, displaying greater affinity for serotonin $5HT_2$ receptors relative to dopamine D_2 receptors [8].

The promise of atypical antipsychotics took a hit early in the 1970s when the early clinical use of clozapine was marked by a devastating clinical trial in Finland in which 17 patients (of about 3,000) developed agranulocytosis (a blood condition with reduced white blood cells) and eight of those patients died [9]. None-the-less, clozapine was eventually approved in the USA by the FDA on September 26, 1989 for use in treatment-resistant schizophrenia ([6]; www.accessdata.fda.gov). The introduction of clozapine for clinical use stimulated the development of a large number of these newer "atypical" (second-generation) antipsychotic drugs for the treatment of schizophrenia over the next 25 years; an initiative that greatly reduced EPS liability during treatment (although there are other significant side effects). Overall, there are advantages and disadvantages to the implementation of either atypical antipsychotic or typical antipsychotic, treatment strategies (e.g., [10]). Given the current state of medicinal chemistry, neuropsychopharmacology, and behavioral and molecular neuroscience, newer treatment strategies for schizophrenic and psychotic disorders are most certainly on the horizon, and drug discrimination will play a critical role in determining their pharmacotherapeutic efficacy and provide a better understanding of their in vivo behavioral effects.

2 Discriminative Stimulus Properties of Antipsychotic Drugs

Drug discrimination assays are useful in that they assess the subjective effect of a drug, usually referred to as a compound's "*discriminative cue*" or "*discriminative stimulus*" for that drug. Given that drugs belonging to similar therapeutic and/or behavioral classifications often share the same subjective effects in humans, as in other animals, drug discrimination studies offer a unique opportunity for translational

approaches in nonhuman experimental preparations. Quantitatively, drug discrimination can be used to classify existing drugs and novel compounds and it can be further used to relate those subjective effects to specific receptor mechanisms in the brain. While there is clearly more research with drugs of abuse utilizing the drug discrimination paradigm, there is a substantial literature on the discriminative stimulus properties of psychotherapeutic drugs used in humans (e.g., see Chapter 11 – *The Discriminative Stimulus Properties of Drugs Used to Treat Depression and Anxiety* in this volume; [11]). The focus of the present chapter is on antipsychotic drugs and we provide a brief summary of the drug discrimination literature on antipsychotic drugs below, but we do not intend to review all of the literature in this field as comprehensive reviews are already available [12, 13]. Instead, this chapter will focus on the *translational value* of drug discrimination as a preclinical assay for drug development. The definition of translational research is somewhat vague and exactly how it differs from basic and applied research is debatable (see Fang and Casaderall [14]). In this chapter (and in Chapter 4 – Cross-species translational findings in the discriminative stimulus effects of ethanol) the term translation research is more focused on the ability to translate nonhuman animal studies findings to human studies and/or to the prevention or treatment of human disease. It should also be noted that the actual translation value of any basic research may not be known for years. As you will see in the sections to follow, the present chapter will focus on the role of training dose and cross-species comparisons.

The typical antipsychotic chlorpromazine was first tested in a discrimination task (three-compartment test chamber similar to a T-maze) by Stewart [15]. In this study, rats were trained to discriminate chlorpromazine (4.0 mg/kg, i.p.) from saline and it was found that other phenothiazines fully substituted for chlorpromazine. Barry et al. [16] were the first to successfully establish *two-lever* drug discrimination with 1.0 mg/kg chlorpromazine (i.p.) and found that non-brain penetrant quaternary chlorpromazine did not substitute for chlorpromazine, providing one of the first demonstrations that the discriminative stimulus properties of drugs are centrally mediated. Goas and Boston [17] later reported that 2.0 mg/kg chlorpromazine (p.o.) fully generalized to both the typical antipsychotic haloperidol and the atypical antipsychotic clozapine. More recently, Porter et al. [18] demonstrated that the chlorpromazine discriminative stimulus (1.0 mg/kg, i.p. in rats) generalized fully to the atypical antipsychotics clozapine and olanzapine and to the typical antipsychotic thioridazine, but only partially to the typical antipsychotic haloperidol and the dopamine D_2 antagonist raclopride. Chlorpromazine also has been used in a *three-lever* drug discrimination study by Porter et al. [19] in which rats were trained to discriminate between 5.0 mg/kg clozapine versus 1.0 mg/kg chlorpromazine versus vehicle. Interestingly, in that study the atypical antipsychotic clozapine substituted fully for the typical antipsychotic chlorpromazine at a lower ED_{50} (0.103 mg/kg) before the rats shifted to the clozapine lever to produce full generalization to clozapine ($ED_{50} = 1.69$ mg/kg). In contrast, chlorpromazine did not substitute for clozapine and engendered only chlorpromazine-appropriate responding ($ED_{50} = 0.196$ mg/kg) – thus, replicating the asymmetrical generalization between clozapine and chlorpromazine reported by Goas and Boston [17]. The atypical antipsychotic olanzapine had a

pattern of substitution similar to clozapine. The typical antipsychotic haloperidol produced chlorpromazine-appropriate responding (ED_{50} = 0.007 mg/mg) only. These results confirmed that there is an overlap between the discriminative stimulus properties of clozapine and chlorpromazine, but not between clozapine and haloperidol; whereas, chlorpromazine and haloperidol share overlapping discriminative stimulus properties. These findings suggested that stimulus properties may be similar in some ways, but not identical, between typical and atypical antipsychotic drugs. Given that the subjective effects of drugs are mediated by specific receptor actions, it is not too surprising that clozapine and chlorpromazine share discriminative stimulus properties since their binding profiles are more similar than are clozapine's and haloperidol's binding profiles [20–23].

The typical antipsychotic haloperidol has proven to be much more difficult to establish as a discriminative stimulus. Colpaert et al. [24] trained four rats to discriminate 0.02 mg/kg haloperidol (s.c.); however, it required over 80 training sessions and no other drugs were tested for substitution. McElroy et al. [25] trained rats to discriminate 0.05 mg/kg haloperidol (i.p.) and reported that the phenothiazine chlorpromazine fully substituted for haloperidol and that amphetamine blocked haloperidol's discriminative stimulus indicating that dopamine antagonism was the underlying pharmacological mechanism for haloperidol's discriminative stimulus. One reason for the lack of studies on the discriminative stimulus properties of haloperidol is that it is a very difficult drug to establish as a discriminative cue versus saline. However, it has been used in drug–drug, two-lever discrimination studies (e.g., [26, 27]) and in drug–drug–vehicle, three-lever discrimination (e.g., [28, 29]) primarily to contrast dopamine antagonist with dopamine agonist receptor mechanisms. Thus, drug discrimination studies with typical antipsychotic drugs as the discriminative stimulus are fairly limited and have not proven to be very useful as a behavioral assay for drug discovery; instead, these studies focused on receptor mechanisms underlying their discriminative stimulus properties. In contrast, drug discrimination studies with atypical antipsychotic drugs are more prevalent and have more potential as useful assays for drug discovery.

As described above, clozapine was first established as a discriminative stimulus by Goas and Boston [17]. Later studies suggested that cholinergic receptor antagonism played an important role in the discriminative stimulus properties for clozapine in rats [30–33]; however, other studies suggested that serotonergic mechanisms in pigeons [34] and in C57BL/6 mice [35] mediated clozapine's discriminative cue. In addition, the training dose for clozapine in rats has been shown to be an important factor in drug discrimination studies [36–38]. Further, the panoply of receptors that clozapine (and most other atypical antipsychotics) binds to, coupled with a lack of clozapine substitution by ligands selective for these receptors, led to the notion that clozapine possesses a complex/compound discriminative cue involving multiple receptor actions [13, 31, 38, 39]. The importance of each of these factors (i.e., training dose and species) with clozapine drug discrimination will be more fully explored in sections that follow.

3 Importance of Training Dose for the Discriminative Stimulus Properties of Clozapine in Rats

A promising aspect to preclinical assays is their potential for aiding in drug discovery efforts (e.g., [40, 41]). A large number of drug discrimination studies have been conducted with the atypical antipsychotic drug clozapine. As reviewed by Porter and Prus [13], clozapine drug discrimination in rats has typically used a training dose of 5.0 mg/kg. In the many studies that have used this training dose, the ability of clozapine drug discrimination to distinguish typical from atypical antipsychotic drugs has been mixed and this assay does not appear to be reliably predictive of clozapine's "atypicality" with regard to its antipsychotic effects. Thus, use of a 5.0 mg/kg training dose with clozapine has proven to be of limited utility in terms of identifying the "atypical" characteristics of other antipsychotic drugs and for the development of novel atypical antipsychotic drugs. In contrast, clozapine drug discrimination with a lower training dose appears to be a more sensitive assay for distinguishing atypical from typical antipsychotic drugs (see also Lieberman et al. [42]).

It has been well established in the literature that the training dose of a drug influences its discriminative stimulus properties, and a lower training dose will typically increase sensitivity to the training drug as reflected by a lower ED_{50} value and a leftward shift of the generalization curve (see review by Stolerman et al. [43]). An early example of this was shown in a drug discrimination study with rats trained to discriminate either 1.75 or 5.6 mg/kg morphine from saline [44]. The lower morphine training dose produced approximately a 1/2 log unit leftward shift in the dose–response curve as compared to the higher, 5.6 mg/kg training dose. A dose of 0.3 mg/kg morphine produced over 90% morphine-appropriate responding in the 1.76 mg/kg training dose group; whereas, a dose of 1.0 mg/kg was required in the 5.6 mg/kg training dose group. A similar separation between the dose–response curves was evident when the narcotic antagonist naloxone (1.0 mg/kg) was coadministered with morphine and produced marked rightward shifts in both dose–response curves. In a study with the atypical antipsychotic drug clozapine, Goudie et al. [37] trained two groups of rats to discriminate either 2.0 mg/kg clozapine or 5.0 mg/kg clozapine from vehicle. As expected, the lower clozapine training dose engendered a significant leftward shift, as the ED_{50} was 0.58 mg/kg for the 2.0 mg/kg training dose and was 1.41 mg/kg for the 5.0 training dose (a 2.4-fold shift in the dose–response curve). They found that the atypical antipsychotic zotepine fully substituted to the 2.0 mg/kg training dose of clozapine but produced only a maximum of 50% CLZ-appropriate responding in the 5.0 mg/kg training dose group.

This increased sensitivity of lower training doses also is evident in a *three*-lever drug discrimination study in which rats were trained to discriminate 1.25 mg/kg clozapine (CLZ) from 5.0 mg/kg CLZ from vehicle [45, 46]. As can be seen in Fig. 1, the 1.25 mg/kg CLZ training dose produced a *significant* leftward shift in the dose–response curve (ED_{50} = 0.08 mg/kg; 95% CI = 0.04–0.16 mg/kg) as compared to the 5.0 mg/kg CLZ training dose–response curve (ED_{50} = 2.67 mg/kg; 95%

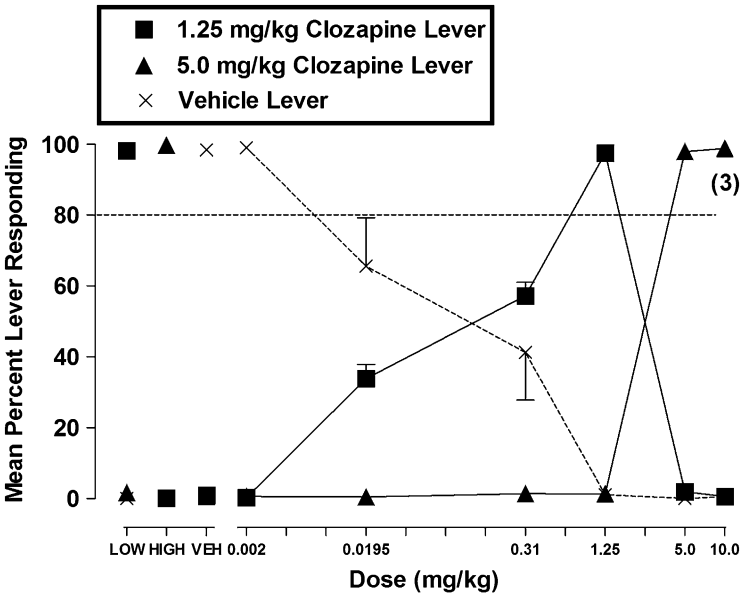

Fig. 1 Rats were trained in a three-lever drug discrimination procedure to discriminate 1.25 mg/kg clozapine (CLZ) from 5.0 mg/kg clozapine from vehicle (VEH). Mean percentage CLZ-lever responding (±SEM) for both training doses is shown on the *y*-axis. Full stimulus generalization was defined as equal to or greater than 80% CLZ-lever responding (*dashed line*). The number in *parenthesis* for the 10 mg/kg CLZ dose indicates the number of rats that met the response rate criteria (i.e., at least five responses per minute) and were therefore included in the mean percentage CLZ-lever responding calculation for that dose. For all other doses, $n = 12$ rats ([46]; reproduced with permission)

CI = 2.45–2.93 mg/kg). In the Prus et al. [45] study, it was found that the atypical antipsychotic olanzapine fully substituted for the 5.0 mg/kg CLZ training dose, but not for the 1.25 mg/kg CLZ training dose. In contrast, the atypical antipsychotics quetiapine and sertindole produced full substitution for the 1.25 mg/kg CLZ training dose, but not for the 5.0 mg/kg CLZ training dose. Similarly, the atypical risperidone produced strong, partial substitution (72% CLZ-appropriate responding) for the 1.25 mg/kg CLZ training dose and failed to substitute for the 5.0 mg/kg CLZ training dose.

The suggestion that training dose was an important factor for clozapine's discriminative stimulus was first demonstrated by Goudie et al. [37]. In an abstract, they reported the results of a study in which rats were trained to discriminate either 2.0 mg/kg CLZ or 5.0 mg/kg CLZ from vehicle. They found that generalization curves for clozapine, olanzapine, quetiapine, and JL13 (a clozapine congener) were all shifted to the left in the lower 2.0 mg/kg training dose group reflecting greater sensitivity for the lower clozapine training dose. Porter et al. [38] further examined the utility of using a lower clozapine training dose to determine if low-dose clozapine drug discrimination could distinguish typical and atypical antipsychotic drugs. In rats

trained to discriminate 1.25 m/kg clozapine from vehicle, it was found that the atypical antipsychotics risperidone, sertindole, and olanzapine fully substituted (i.e., >80% CLZ-appropriate responding) for the 1.25 mg/kg training dose of clozapine, although partial substitution (i.e., >60% CLZ-appropriate responding) occurred with the atypical quetiapine (as noted above, Goudie et al. [37] reported full substitution with a 2.0 mg/kg CLZ training dose). In contrast, the typical antipsychotics haloperidol, chlorpromazine, and fluphenazine did not engender CLZ-appropriate responding (although it should be noted that thioridazine did produce partial substitution for clozapine). In another low-dose 1.25 mg/kg clozapine study, full substitution was shown for the atypical antipsychotic melperone [47]. Thus, a number of studies using lower training doses of clozapine in rats (1.25 or 2.0 mg/kg) have found that clozapine's discriminative stimulus generalizes to a greater number of atypical antipsychotic drugs than when a higher clozapine training dose (i.e., 5.0 mg/kg) is used. Thus, low-dose clozapine drug discrimination in rats has greater translational value than high-dose clozapine drug discrimination for development of new antipsychotic drugs. In the next section, we examine the underlying neuropharmacological mechanisms that mediate the discriminative stimulus properties of the 5.0 and 1.25 mg/kg training doses for clozapine in rat drug discrimination studies.

3.1 Neuropharmacological Mechanisms Mediating the Clozapine Discriminative Stimulus

3.1.1 5.0 mg/kg Clozapine Training Dose in Rats

Clozapine has a very diverse binding profile and differs from typical antipsychotics like haloperidol in that it displays a higher binding affinity for serotonin 5-HT_2 receptors than for dopamine D_2 receptors (see Meltzer et al. [8]). However, clozapine also has significant affinity for a number of other receptors including dopaminergic D_4, serotonergic 5-$HT2_C$, 5-HT_6, 5-HT_7, cholinergic M_1, M_2, M_3, M_4, adrenergic α_1, α_2, and histamine H_1 receptors [20–23]. A number of studies have suggested that clozapine has a compound (complex) discriminative stimulus that is mediated by its activity at several of these receptors (see review by Porter and Prus [13]). However, in rats trained to discriminate 5.0 mg/kg clozapine the one receptor mechanism that has consistently emerged as important for mediating clozapine's discriminative cue is antagonism of muscarinic cholinergic receptors. Cholinergic antagonism was first proposed by Nielsen [33] who reported that scopolamine and atropine substituted for clozapine (5.76 mg/kg training dose). Kelley and Porter [32] found that there was a significant correlation ($r = 0.74$, $p < 0.01$) between the percentage of clozapine-appropriate lever responding and the percentage of scopolamine-appropriate lever responding in rats trained to discriminate either 5.0 mg/kg clozapine or 0.125 mg/kg scopolamine from vehicle (see Fig. 2).

The fact that clozapine and scopolamine displayed cross-generalization (i.e., both produced full substitution for the other drug) suggests that a common mechanism of

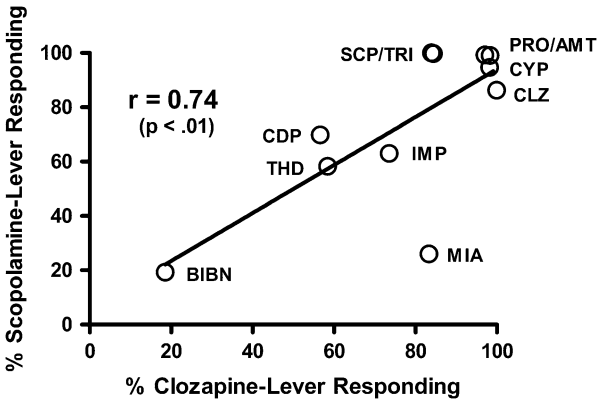

Fig. 2 Summary of results for drugs tested in rats trained to discriminate 5.0 mg/kg (i.p.) from vehicle and in rats trained to discriminate 1.25 mg/kg scopolamine from vehicle in two-lever drug discrimination. The highest percent of scopolamine-lever responding (*y*-axis) is shown as a function of the highest percent of clozapine-lever responding (*x*-axis) for each drug. The regression line for the data and the correlation coefficient also are shown. Abbreviations: *AMT* amitriptyline; *BIBN* BIBN-99; *CDP* chlordiazepoxide; *CLOZAPINE* clozapine; *CYP* cyproheptadine; *IMP* imipramine; *MIA* mianserin; *PMZ* promethazine; *SCP* scopolamine; *THD* thioridazine; *TRI* trihexyphenidyl (data adapted from Kelley and Porter [32] and published in Porter and Prus [13]; reproduced with permission)

action was shared by them (i.e., antagonism of cholinergic muscarinic receptors). Also, it was found that antagonism of cholinergic muscarinic M_1 receptors was more important than antagonism of M_2 receptors as the preferential M_1 muscarinic antagonist trihexyphenidyl fully substituted for clozapine and scopolamine; whereas, the M_2 muscarinic antagonist BIBN 99 did not substitute for either drug. These findings strongly suggest that antagonism of muscarinic cholinergic receptors (probably M_1) mediates the discriminative stimulus properties of 5.0 mg/kg clozapine. Antagonism of muscarinic receptors also has been shown in other studies to be important for clozapine's discriminative cue at this higher training dose [31, 47].

3.1.2 1.25 mg/kg Clozapine Training Dose in Rats

As discussed above, there are clear differences in the discriminative stimulus properties of 5.0 mg/kg clozapine and 1.25 mg/kg clozapine as reflected by the ability of these two different training doses to distinguish between typical and atypical antipsychotic drugs. While the discriminative cue of the 5.0 mg/kg training dose in rats appears to involve antagonism of muscarinic cholinergic receptors, different mechanisms appear to be responsible for the discriminative cue of the lower 1.25 mg/kg training dose. Recently, Prus et al. [48] examined the mechanisms underlying the discriminative stimulus properties of 1.25 mg/kg clozapine in a two-lever drug discrimination study in rats by testing a number of selective ligands. They found evidence for a compound/complex discriminative cue, as selective ligands at several receptors

produced full or partial substitution for 1.25 mg/kg clozapine. Specifically, the 5-HT_{2A} inverse agonist M100907 and the two preferential $D_{4/5}$-HT_2/α_1 receptor antagonists Lu37-114 and Lu37-254, and the α_1 adrenoceptor antagonist prazosin fully substituted for clozapine's discriminative stimulus. These findings suggest that serotonin 5-HT_{2A} inverse agonism and antagonism of dopamine D_4 and noradrenergic α_1 receptor antagonism mediate the discriminative stimulus properties of 1.25 mg/kg clozapine in rats and further confirm that clozapine produces a complex, compound discriminative stimulus.

These differences in the underlying neuropharmacological mechanisms that mediate the discriminative stimulus properties of clozapine at low and high training doses appear to be the primary factor in the ability of other antipsychotic drugs to substitute fully or partially for clozapine. While additional research will be necessary to determine the actual utility of using a low clozapine training dose for development of novel antipsychotic drugs, the research discussed above demonstrates the necessity of considering important controlling variables in the drug discrimination procedure (and of course all behavioral assays used for drug development). It may be that cholinergic mechanisms at the higher training dose are more related to the nontherapeutic properties of clozapine (and other atypical antipsychotics) than are the serotonergic, dopaminergic, and adrenergic mechanisms evident at the lower training dose. In the following sections, we will discuss another important variable for the translational value of clozapine drug discrimination – differences in the discriminative stimulus properties of clozapine across different species of animals.

4 Importance of Species Similarities/Differences for the Translational Value of Clozapine Drug Discrimination

As discussed in the preceding sections, training dose is an important variable for the translational value of the clozapine drug discrimination assay in rats. What is the translational value of clozapine drug discrimination in other species of animals? The vast majority of clozapine drug discrimination studies have been conducted with rats as the subjects. Using information from a review by Porter and Prus in 2009, a total of 34 articles were reviewed in which an antipsychotic was used as the training drug and 26 of those used clozapine. Only five of those studies used a species other than rats. One study used squirrel monkeys [49], one study used pigeons [34], two studies used C57BL/6 mice [35, 50], and one study used DBA/2 mice [51]. Since 2009, a PubMed/Medline search found three additional studies that did not use rats as the subjects in clozapine drug discrimination studies. Two of those studies used C57BL/6 mice [52, 53] and one used hybrid B6129 mice [54]. Below we will review and compare the similarities and differences of clozapine's discriminative stimulus across these different species and include some recent findings from our laboratory.

Pigeons were trained by Hoenicke et al. [34] to discriminate 1.0 mg/kg clozapine (i.m.) from saline in a two-key drug discrimination procedure. They reported that test compounds with antagonist activity at 5-HT_2 receptors fully substituted for clozapine's discriminative stimulus. This included the drugs cyproheptadine (antihistamine), metergoline (psychoactive drug of the ergoline chemical class), mianserin (tetracyclic antidepressant), pizotifen (benzocycloheptene-based drug used for treating migraines), and fluperlapine (atypical antipsychotic). Hoenicke et al. also tested a large number of selective receptor ligands that were active at non-serotonergic systems and compounds from other therapeutic classifications and none of them substituted for clozapine. They also tested the typical antipsychotics chlorpromazine and thioridazine, neither of which substituted for clozapine. Unfortunately, they did not test other atypical antipsychotic drugs, as there was limited availability of such compounds at the time this study was conducted with pigeons. This finding obviously was in contrast to the earlier suggestion by Nielsen [33] that clozapine's discriminative stimulus in rats was mediated by antagonism of muscarinic cholinergic receptors for higher clozapine training doses (however, serotonergic mechanisms are more important at a low clozapine training dose in rats; see discussion above in Sect. 4.1.2). Also, Nielsen's study was with rats and Hoenicke et al.'s study was with pigeons. This was the first indication that species was an important variable to consider when studying the pharmacological mechanisms underlying the discriminative stimulus properties of clozapine and the possible utility of clozapine drug discrimination for development of novel antipsychotic drugs.

Carey and Bergman [49] trained squirrel monkeys to discriminate 1.0 mg/kg clozapine (i.m.) from saline in a two-lever drug discrimination procedure. The typical antipsychotics haloperidol, chlorpromazine, and thioridazine produced only minimal clozapine-appropriate responding (maximum of 44% clozapine-lever responding by 0.3 mg/kg chlorpromazine). Higher doses of these three drugs produced marked rate suppression. In contrast, the atypical antipsychotic drugs fluperlapine and quetiapine and the structurally related dibenzothiophene, perlapine all produced greater than 90% clozapine-appropriate responding. The atypical antipsychotics olanzapine and risperidone failed to substitute for clozapine, producing significant response rate suppression. However, when the dopamine D_2 agonist (+)-PHNO was coadministered with olanzapine, greater than 90% clozapine-appropriate responding was evident. Thus, clozapine drug discrimination in squirrel monkeys was able to distinguish atypical from typical antipsychotic drugs, albeit with some false negatives that may be attributed to the dopaminergic rate suppressant effects of higher doses for some of the antipsychotic drugs. Unfortunately, Carey and Bergman did not explore the underlying pharmacological mechanisms that mediate clozapine's discriminative stimulus in squirrel monkeys. The following section examines the discriminative stimulus properties of the atypical antipsychotic clozapine in several inbred mouse strains.

4.1 Discriminative Stimulus Properties of Clozapine in Mice

4.1.1 C57BL/6 Mice

The first study to examine the discriminative stimulus properties of clozapine in mice was by Philibin et al. [35]. In that study, male C57BL/6 mice were trained to discriminate 2.5 mg/kg (s.c.) from vehicle in a two-lever procedure (attempts to train 5.0 mg/kg clozapine were unsuccessful due to response rate suppression). Thirteen of 20 mice successfully acquired the clozapine discriminative cue and completed the clozapine dose–response curve with an $ED_{50} = 1.14$ mg/kg. It was shown that the serotonergic 5-$HT_{2A/2B/2C}$ receptor antagonist ritanserin fully substituted for clozapine's discriminative stimulus and that the 5-HT_2 agonist quipazine produced a significant attenuation of clozapine's cue. Other selective ligands tested included the muscarinic receptor antagonist scopolamine, which produced partial substitution for clozapine (62% clozapine-appropriate responding). However, the indirect dopamine agonist amphetamine and the 5-HT_2 agonist quipazine did not produce clozapine-appropriate responding. These findings demonstrated that antagonism of serotonergic 5-HT_2 receptors is an important pharmacological mechanism underlying the clozapine discriminative cue in C57BL/6 mice. This finding was confirmed in a subsequent study in which male C57BL/6 mice also were trained to discriminate 2.5 mg/kg (s.c.) clozapine from vehicle [50]. The selective 5-HT_{2A} inverse agonist M100907 fully substituted for clozapine. In addition, the α_1-adrenoceptor antagonist prazosin also fully substituted for clozapine's discriminative stimulus. Thus, antagonism (or inverse agonism) of *either* serotonin 5-HT_2 receptors *or* α_1-adrenoceptors is sufficient to produce clozapine-appropriate responding in male C57BL/6 mice. Interestingly, it is also possible for drugs to share partial discriminative stimulus properties, likely through indirect mechanisms. Vunck et al. [52] demonstrated this in male C57BL/6 mice trained to discriminate either 2.5 mg/kg clozapine or 30 mg/kg N-methyl-D-aspartate (NMDA, glutamatergic agonist) from vehicle. Cross-generalization testing found partial substitution of clozapine for NMDA, but none for NMDA in clozapine-trained mice. However, when a non-generalizing dose of each training drug was tested in combination with the other drug, there was full and dose-dependent substitution in both groups (i.e., cross-generalization). Interesting, the α_1-adrenoceptor antagonist prazosin fully substituted for both clozapine and NMDA suggesting that any shared discriminative stimulus properties for clozapine and NMDA were likely mediated through α_1 adrenergic antagonism.

4.1.2 DBA/2 Mice

The discriminative stimulus properties of clozapine in male DBA/2 mice were compared to C57BL/6 mice by Porter et al. [51]. In contrast to C57BL/6 mice, antagonism of serotonergic 5-HT_2 receptors and α_1-adrenoceptors did *not* produce clozapine-appropriate responding. However, as shown in C57BL/6 mice, the muscarinic antagonist

scopolamine produced partial substitution for clozapine (69% clozapine-appropriate responding). Interestingly, haloperidol, which is a potent dopamine antagonist, also produced partial substitution for clozapine in DBA/2 mice (68% clozapine-appropriate responding), but not in C57BL/6 mice (maximum of 48% clozapine-appropriate responding). Differences in brain dopamine systems between DBA/2 and C57BL/6 mice might account for the different substitution patterns of haloperidol. In support of this suggestion, it also was found that a higher dose of haloperidol (0.4 mg/kg) was required to fully suppress lever pressing in the DBA/2 mice as compared to C57BL/6 mice (0.2 mg/kg) (see Porter et al. [51] for fuller discussion).

4.1.3 129S Mice

In an unpublished study from our laboratory (Webster and Porter; data presented at meetings for the Society for Neuroscience [55, 56] and the European Society for Behavioural Pharmacology [57]), male 129S2 mice were trained to discriminate 1.25 mg/kg (s.c.) clozapine from saline. Initial attempts to train a 2.5 mg/kg dose of clozapine had to be abandoned because of response rate suppression. While the serotonergic 5-HT_2 receptor antagonist ritanserin did not substitute for clozapine (57% clozapine-appropriate responding), the more selective 5-HT_{2A} inverse agonist M100907 produced partial substitution for clozapine (69% clozapine-appropriate responding). The α_1-adrenoceptor antagonist prazosin fully substituted for clozapine (83% clozapine-appropriate responding), which is similar to results observed in C57BL/6 mice, but not in DBA/2 mice. The muscarinic antagonist scopolamine occasioned partial substitution (68% clozapine-appropriate responding), similar to that seen in both C57BL/6 and DBA/2 mice. The dopamine antagonist haloperidol produced partial substitution (66% clozapine-appropriate responding), similar to that seen in DBA/2 mice. Thus, 129S2 mice displayed both similarities to and differences from C57BL/6 and DBA/2 mice. Also, 129S2 mice were clearly more sensitive to the rate-suppressing effects of clozapine as a lower training dose had to be used (DBA/2 mice were less sensitive – see previous section). Thus, some of the differences in substitution patterns for these selective ligands may reflect training dose effects, but this clearly was not the case for C57BL/6 and DBA/2 mice as both were trained at the 2.5 mg/kg dose of clozapine. Thus, the differences between these three inbred strains of mice can probably be attributed primarily to strain differences as opposed to training dose differences.

4.1.4 B6129S Hybrid Mice

A small number of male B6129S hybrid mice have recently been trained to discriminate 1.25 mg/kg clozapine (s.c.) from saline (Webster and Porter, unpublished data). Interestingly, the only selective ligand that produced full substitution for clozapine was the muscarinic antagonist scopolamine (86% clozapine-appropriate responding). The serotonergic 5-HT_2 receptor antagonist ritanserin (37% clozapine-

appropriate responding) and the 5-HT2A inverse agonist M100907 did not substitute for clozapine. However, the α_1-adrenoceptor antagonist prazosin generated partial clozapine-appropriate responding (60% clozapine-appropriate responding), as did the dopamine antagonist haloperidol (73% clozapine-appropriate responding). Thus, the hybrid between C57BL/6 and 129S2 mice more closely resembled findings with rats trained to discriminate 5.0 mg/kg clozapine (see discussion in Sect. 3.1). Table 1 provides a summary of the results of substitution testing with selective receptor ligands in the four inbred mouse strains discussed in Sect. 4.1.

5 Subjective Properties of Antipsychotic Drugs in Humans

While research has been conducted on the discriminative stimulus properties of antipsychotic drugs in humans, unfortunately, no studies have used antipsychotics as the training drugs. Instead, antipsychotic drugs have been administered as a test compound in humans trained to discriminate a different substance. For example, Rush et al. [58] examined the effects of the atypical antipsychotic risperidone in humans trained to discriminate D-amphetamine. When coadministered with D-amphetamine,

Table 1 Receptor mechanisms mediating the discriminative stimulus properties of the atypical antipsychotic clozapine in four inbred mouse strains

Receptor mechanism	C57BL/6 mice[a]	DBA/2 mice[b]	129S2 mice[c]	B6129S mice[c]
5-HT$_2$				
Ritanserin (antagonist)	FULL	NO	NO	NO
5-HT$_{2A}$				
M100907 (inverse agonist)	FULL	NT	PARTIAL	NO
α_1 adrenoceptor				
Prazosin (antagonist)	FULL	NO	FULL	PARTIAL
Muscarinic				
Scopolamine (antagonist)	PARTIAL	PARTIAL	PARTIAL	FULL
Histamine H$_1$				
Pyrilamine (antagonist)	NO	NO	NO	NT
Dopamine				
Amphetamine (indirect agonist)	NO	NO	NO	NT
Dopamine D$_2$				
Haloperidol (antagonist)	NO	PARTIAL	PARTIAL	PARTIAL

The training dose for clozapine was 2.5 mg/kg in C57BL/6 and DBA/2 mice and 1.25 mg/kg in 129S2 and B6129S mice

FULL full substitution >80% clozapine-appropriate responding, *PARTIAL* partial substitution >60% to <80% clozapine-appropriate responding, *NO* no substitution <60% clozapine-appropriate responding

[a]Philibin et al. [35, 50]

[b]Porter et al. [51]

[c]Webster and Porter, unpublished data

risperidone (which serves as a D_2 receptor antagonist among other receptor actions) reduced D-amphetamine-appropriate responding from full generalization (>80%) to approximately 40%. The reduction in D-amphetamine-appropriate responding corresponded with related changes in reported subjective effects of D-amphetamine, including participants endorsing fewer words related to stimulant effects, a willingness to take the drug again, feelings of liking the drug, the level of good effects produced by the drug, etc.

In another study from this group, Lile et al. [59] tested the atypical antipsychotic drug aripiprazole in combination with cocaine in humans trained on a cocaine discrimination task. Coadministration of aripiprazole caused fewer doses of cocaine to be statistically greater placebo, suggesting an attenuation of cocaine's discriminative cue. When tested alone, aripiprazole did not engender cocaine-appropriate responding. Aripiprazole differs somewhat from other atypical antipsychotic drugs, in that it acts as a weak partial agonist at dopamine D_2 receptors, rather than as an antagonist. The study by Lile et al. [59] demonstrated that the degree of activation of D_2 receptors produced by aripiprazole alone was not sufficient to produce cocaine-like stimulus effects. Given that cocaine-like stimulus effects rely primarily on D_2 receptor activation, it is not surprising that aripiprazole did not substitute for cocaine as its effects are likely mediated by functional antagonism of D_2 receptors (i.e., the population of D_2 receptors occupied by aripiprazole but not activated by this compound). Changes in the subjective effects for cocaine when combined with aripiprazole were similar to the findings in the Rush et al. [58] study when risperidone was combined with amphetamine, with overall reduced subjective effects.

These studies in humans evaluating the stimulus effects of antipsychotic drugs can be more precisely described as a reference to psychostimulant studies and indicate that antipsychotic stimulus effects counter those produced by psychostimulants. Arguably, if an antipsychotic drug was used during discrimination training, then the discrimination would be based on the subjective (interoceptive) effects of the antipsychotic drug. Further, assessing the stimulus effects of antipsychotic drugs in patient populations for which the drugs were prescribed would provide additional benefits for the scientific value of data gathered on these compounds. Such stimulus effects might consist of or depend on: (1) individual differences in level of impairment, (2) the development of drug side effects, or (3) adaptations, such as tolerance or sensitization, to the interoceptive drug state after multiple administrations.

Moncrieff et al. [60] examined an internet site where users freely post comments on the effects they feel when taking antipsychotic medications. In their analysis of posted comments, the groups compared older antipsychotics (defined, in this case as chlorpromazine, trifluoperazine, or haloperidol) with risperidone and olanzapine. The more prevalent comments for all antipsychotic drugs regarded feelings of sedation, reduced motivation, and flattened emotions. A number of participants noted sexual dysfunction with risperidone, and olanzapine was particularly regarded as causing weight gain and increased appetite. The older antipsychotics were noted more often than risperidone or olanzapine for producing Parkinsonian effects.

As reviewed by Gerlach and Larsen [61], the vast majority of patients given long-term antipsychotic treatment displayed features of extrapyramidal motor symptoms.

These side effects were most prevalent among compounds with a strong affinity for dopamine D_2 receptors, including sulpiride, amisulpride, and risperidone [23]. Sedation was most common among patients treated with clozapine, but also relatively prevalent with olanzapine and quetiapine; all three of which are antagonists at histamine H_1 receptors, which have linked to sedation, although effects at other receptors may contribute to this effect.

Using an assessment of subjective well-being for antipsychotic treatment, Mizrahi et al. [62] correlated regional D_2 receptor occupancy produced by olanzapine or risperidone with total scores on the assessment. They found that patients indicated a diminished sense of overall well-being that negatively correlated with higher D_2 receptor occupancy in the striatum, temporal lobe, and insular cortex. An evaluation of the assessment's subscales indicated correlations between striatal D_2 receptor occupancy and mental functioning and between temporal lobe D_2 receptor occupancy and emotional regulation.

Thus, overall, only a limited number of studies have examined the discriminative stimulus properties of antipsychotic drugs in humans. Studies that have examined antipsychotic drugs tend to indicate that these drugs counteract the effects of psychostimulant drugs that cause the activation of dopamine receptors, which would be in keeping with the tendency of antipsychotic drugs to act as D_2 receptor antagonists [23]. Many of the self-reported subjective effects of antipsychotic drugs, which presumably would contribute to the discriminative stimulus properties of these drugs in humans, are associated with side effects. Reasonable links to receptor actions can be made to these subjective side effects in humans, with typical antipsychotic drug effects most often associated with D_2 receptor antagonism; whereas, atypical antipsychotic drug effects are linked to D_2 receptor antagonism along with other receptor mechanisms [23, 61]. For example, an adverse effect not described above but which offers an example of a non-dopaminergic mediated side effect is dry mouth. Dry mouth results from antagonism of cholinergic muscarinic receptors, which is found with some atypical antipsychotic drugs such as clozapine and olanzapine [63, 64].

6 Conclusions

Conclusions from the literature discussed in this chapter are somewhat limited given that only a small number of studies have actually examined clozapine's discriminative stimulus properties in mice, only one study in pigeons, one study in squirrel monkeys, with the rest of the studies using rats as the subject of choice. However, some basic conclusions can be reached about clozapine's discriminative stimulus across different species of animals. First, clozapine clearly has a robust discriminative stimulus as evidenced by the relative ease in establishing it as a training drug in both two- and three-lever drug discrimination paradigms across different species. Second, serotonin receptors appear to be an important pharmacological mechanism mediating clozapine's discriminative cue although differences are clearly evident as antagonism of cholinergic muscarinic receptors is important in rats at a higher training dose (5.0 mg/kg) of

clozapine, but not at a lower training dose (1.25 mg/kg) species and even across inbred mouse strains. Third, in mice antagonism of muscarinic receptors appears to be less important in C57BL/6, DBA/2, and 129S2 mice (produces partial substitution for clozapine); however, it appears to mediate clozapine's cue in hybrid B6129S mice. Fourth, antagonism of α_1 adrenoceptors is a sufficient mechanism in C57BL/6 and 129S2 mice, but not in DBA/2 and B6129S mice, and only produces partial substitution in low-dose clozapine discrimination in rats. Fifth, dopamine antagonism produces partial substitution in DBA/2, 129S2, and B6129S mice, but not in C57BL/6 mice, and partial substitution with D_4 antagonism in low-dose clozapine drug discrimination in rats. Thus, it is evident that clozapine has a complex mixture of receptor contributions towards its discriminative cue based on the four mouse strains that have been tested, similar to the results from rat studies. Finally, we find evidence that antipsychotics produce subjective effects in humans that can be predicted from animal studies, although the limited number of studies examining discriminative stimuli of antipsychotics in humans makes this conclusion tentative.

While the majority of research has focused on rats, drug discrimination studies with mice offer an interesting opportunity for future research and hopefully will have greater translational value for future drug development, since the majority of genetic manipulations are in mice – not rats. Given the advances of using mice for behavioral pharmacogenetic approaches, an important first step is to establish behavioral, pharmacological, physiological, and biochemical comparisons across different inbred mouse strains. C57BL/6 and DBA/2 mice represent the most commonly used inbred mouse strains in behavioral pharmacology research (see Crawley et al. [65] for review) and they are important background strains for developing transgenic and knockout mouse models. The 129S2 and B6129S mouse strains also are frequently used in genetic mouse models. Thus, the research presented here for these four mouse strains represents a first step in establishing a necessary foundation of background information. More research is required to fully characterize the underlying mechanisms that characterize the similarities and differences across these inbred mouse strains and to determine how that relates to functional and anatomical characteristics (e.g., there are known differences in brain dopamine systems in C57BL/6 and DBA/2 mice; see Porter et al. [51] for fuller discussion). Hopefully, better characterization of these background mouse strains will aid in future drug development efforts for new and better antipsychotic medications. A further examination of antipsychotic stimulus properties in humans, particularly in patients with schizophrenia, would go far in evaluating the translational value of the drug discrimination paradigm for antipsychotic drugs.

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The Discriminative Stimulus Properties of Drugs Used to Treat Depression and Anxiety

Adam J. Prus and Joseph H. Porter

Abstract Drug discrimination is a powerful tool for evaluating the stimulus effects of psychoactive drugs and for linking these effects to pharmacological mechanisms. This chapter reviews the primary findings from drug discrimination studies of antidepressant and anxiolytic drugs, including novel pharmacological mechanisms. The stimulus properties revealed from these animal studies largely correspond to the receptor affinities of antidepressant and anxiolytic drugs, indicating that subjective effects may correspond to either therapeutic or side effects of these medications. We discuss drug discrimination findings concerning adjunctive medications and novel pharmacologic strategies in antidepressant and anxiolytic research. Future directions for drug discrimination work include an urgent need to explore the subjective effects of medications in animal models, to better understand shifts in stimulus sensitivity during prolonged treatments, and to further characterize stimulus effects in female subjects. We conclude that drug discrimination is an informative preclinical procedure that reveals the interoceptive effects of pharmacological mechanisms as they relate to behaviors that are not captured in other preclinical models.

Keywords Animal models • Antidepressant • Anxiety • Anxiolytic • Depression • Discriminative stimulus • Drug discrimination • Operant • Preclinical model • Sedative • Stimulus properties

A.J. Prus (✉)
Department of Psychology, Northern Michigan University, Marquette, MI, USA
e-mail: aprus@nmu.edu

J.H. Porter
Department of Psychology, Virginia Commonwealth University, Richmond, VA, USA

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Contents

1 Introduction

This chapter, located within the volume *The Behavioural Neuroscience of Drug Discrimination* as part of the *Current Topics in Behavioral Neurosciences* series, pertains to the utilization of drug discrimination as a way of examining the stimulus properties of antidepressants and anxiolytics. In this chapter, we review the major findings from the experimental literature to describe the current state of knowledge on the discriminative stimulus properties of these pharmacotherapeutics.

2 Treatments for Depression and Anxiety

The antitubercular drug iproniazid was the first serendipitously discovered compound to have antidepressant efficacy. Synthesized in the early 1950s [1], iproniazid's mechanism of actions included inhibition of monoamine oxidase (MAO) [2]. A series of studies evaluating iproniazid for treating tuberculosis reported improvements in mood, later leading to reports of reduced symptoms in depressed patients. While iproniazid was first marketed only as an antitubercular drug, it was swiftly incorporated as an off-label treatment for clinical depression. Because iproniazid was an irreversible inhibitor of both MAO_A and MAO_B [3], its clinical

utility as an antidepressant was limited due to side effects, including hypertension and digestive complications. Modern MAO inhibitors include selective inhibitors of MAO_B, which is found in the central nervous system (CNS), or reversible inhibitors of MAO_A, which is found in brain and outside the CNS, including the liver and gastrointestinal tract (for review, see [4]).

The development of tricyclic antidepressants arose from efforts to replicate the success of chlorpromazine for the treatment of psychosis. Imipramine was synthesized from promethazine, a compound structurally similar to chlorpromazine. Imipramine proved ineffective for psychosis, but reductions in depressive symptoms in patients with depressive psychosis led to the notion of using imipramine as a novel antidepressant drug (see [4, 5]).

The pharmacological mechanisms of action for tricyclic antidepressant drugs are heterogeneous. Most tricyclic antidepressants inhibit reuptake of serotonin and norepinephrine, but have a weak affinity for dopamine transporters. Tricyclic antidepressant drugs also act as antagonists with a high affinity for the histamine H_1 receptor, and with a moderate affinity for serotonin $(5\text{-HT})_{2A}$, 5-HT_{2C}, and muscarinic receptors [6–10].

The success of tricyclic antidepressant drugs for treating depression led to development of zimelidine, a selective serotonin reuptake inhibitor (SSRI), by Astra AB Pharmaceuticals [11]. Marketed under the trade name Zelmid® in 1982, adverse side effects led Astra to remove zimelidine from the European market in 1983, paving the way for the development of fluoxetine by Eli Lilly. Fluoxetine and other SSRIs are more selective for the serotonin transporter versus the dopamine or norepinephrine transporter, but some SSRIs exhibit appreciable binding affinities for orthosteric monoamine neurotransmitter receptors. For example, fluoxetine exhibits a moderate affinity for 5-HT_{2A} receptors and a relatively high affinity for 5-HT_{2C} receptors [12]. Also, the SSRI paroxetine binds with high affinity for the serotonin transporter and has a moderate affinity for the norepinephrine transporter, and in addition, paroxetine binds with a moderate affinity for muscarinic cholinergic receptors [7]. On the other hand, the SSRI escitalopram exhibits selectivity for the serotonin transporter with a low affinity for monoamine receptors [13].

Many antidepressant drugs exhibit a high affinity for both serotonin and norepinephrine transporters, thus leading to the development of a combined serotonin–norepinephrine reuptake inhibitor (SNRI) as a promising line of antidepressant action. The first antidepressant to be marketed as an SNRI was venlafaxine by Wyeth in 1993. Receptor binding studies show a moderate to weak binding affinity of venlafaxine for the norepinephrine transporter and an approximately eightfold preference for the serotonin transporter [7, 12, 14]. Desvenlafaxine, the active metabolite of venlafaxine, has a similar binding profile and was later approved as an SNRI antidepressant drug [15]. Moreover, venlafaxine appears to lack an appreciable affinity for monoamine, and perhaps other types of receptors [12, 14, 16]. The SNRI duloxetine exhibits a high affinity for both serotonin and norepinephrine transporters, with approximately a tenfold greater affinity for the serotonin transporter over the norepinephrine transporter; and it exhibits a moderate affinity

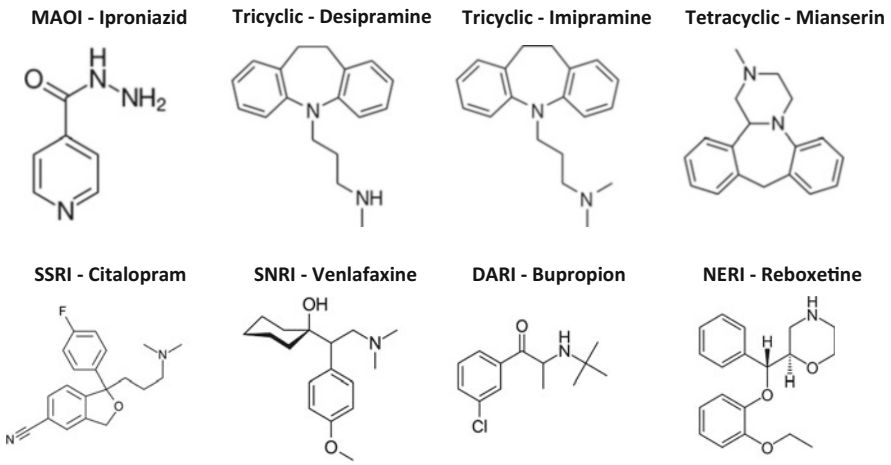

Fig. 1 Structures for antidepressant drugs

for the dopamine transporter and moderate to weak affinities for serotonin 5-HT_{2A} and 5-HT_6 receptors [16].

Newer antidepressants with mechanisms of action that do not fit precisely into the categories described above are often considered as atypical antidepressants, multi-modal antidepressant compounds, or second generation antidepressants. The best known of these more recent and promising compounds is bupropion. Bupropion exhibits a moderate affinity for the dopamine transporter and a weak affinity for the norepinephrine and serotonin transporters as well as monoamine neurotransmitter receptors [12]. Reboxetine is also a selective norepinephrine reuptake inhibitor, although it possesses a modest affinity for serotonin transporters [14], and reboxetine is not usually considered an SNRI given its greater selectivity for the norepinephrine transporter. Vortioxetine exhibits a high affinity for the serotonin transporter, yet possesses a high affinity for a number of serotonin receptors, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{3A}, and 5-HT_7 receptors [17].

Thus, since the discovery of the first antidepressant drug iproniazid, all antidepressants that have been marketed share mechanisms of action that enhance monoamine neurotransmission (see Fig. 1 for examples of drug structures). The most common pharmacological antidepressant action is derived from directly elevating serotonin concentrations in brain, but drugs such as bupropion and reboxetine suggest that directly acting on serotonin neurotransmission may not be a sole requirement for producing antidepressant effects. Many, but not all, of the SSRIs bind to 5-HT_{2C} receptors and various other serotonin receptors. The affinity of tricyclic antidepressant drugs for muscarinic and histamine receptors has traditionally been regarded as unbeneficial for clinical efficacy, and instead the primary cause of side effects, such as dry mouth, blurred vision, and constipation (linked to anticholinergic effects) or sedation (linked to their antihistaminergic effects), although recently more attention has been given to muscarinic antagonism as a

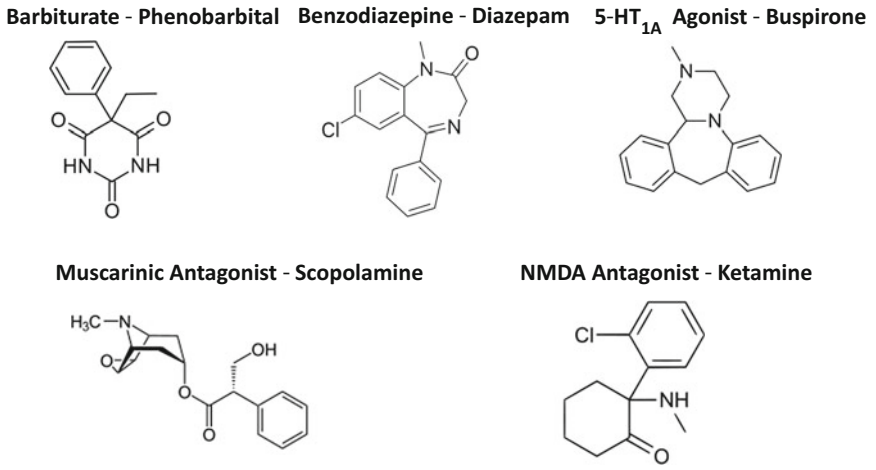

Fig. 2 Structures for antianxiety drugs and other drugs with antidepressant efficacy

possible therapeutic target for treating depression ([18]; see below). Also, glutamatergic targets for the treatment of depression have been the focus of much research since Berman et al. [19] demonstrated that a low, subanesthetic dose (i.v.) of the noncompetitive N-methyl-D-aspartate (NMDA) antagonist ketamine produced rapid and sustained antidepressant effects in depressed patients (for reviews see [5]; [20]).

Drugs that act on a number of different pharmacological mechanisms, including barbiturates, benzodiazepines, serotonin 5-HT_{1A} rector agonists, and most recently antidepressant drugs that inhibit serotonin reuptake have been used to treat anxiety (see Fig. 2 for examples of drug structures). Section 3 will examine the stimulus properties of antidepressant drugs (which include drugs used as a first-line treatment for anxiety) and Sect. 4 will examine the discriminative stimulus properties of barbiturate, benzodiazepine, and serotonin 5-HT_{1A} receptor agonist drug classes. Section 5 will present information on novel therapeutic targets for the treatment of depression, and finally Sect. 6 will provide an overview of adjunctive strategies for the treatment of depression.

3 Discriminative Stimulus Properties of Antidepressant Drugs

3.1 MAO Inhibitors

The first assessment of MAO inhibition in a drug discrimination study was by Huang and Ho [21] who found that the nonselective $MAO_{A/B}$ inhibitor iproniazid failed to substitute for d-amphetamine in rats. However, full substitution did occur

after pretreatment with beta-phenylethylamine, which is a monoamine alkaloid and produces pharmacodynamic effects similar to d-amphetamine. The authors concluded that iproniazid and beta-phenylethylamine in combination had additive effects, together sufficient to mimic d-amphetamine's discriminative stimulus effect.

To our knowledge, only one study has established MAO inhibitors as a training drug in the drug discrimination paradigm. Overton [22] used a T-maze procedure to train the rats to discriminate the stimulus effects of a drug in order to escape a grid floor shock. Overton found that the nonselective MAO_A/MAO_B inhibitors iproniazid, nialamide, phenelzine, and tranylcypromine were readily established as discriminative stimuli (13–27 sessions), while nialamide required greater than 40 sessions to learn the discrimination. In other studies, MAO inhibitors have been evaluated as test compounds to determine if MAO inhibitors would mimic the discriminative stimulus properties of other training drugs like the psychostimulant cocaine. Cocaine, in particular, is useful for these studies, since cocaine enhances dopamine, norepinephrine, and serotonin levels [23–25].

Colpaert et al. [26] reported that the nonselective $MAO_{A/B}$ inhibitor tranylcypromine fully substituted for cocaine's discriminative stimulus in rats. In a subsequent study, these scientists also found that the selective MAO_B inhibitor deprenyl and the nonselective $MAO_{A/B}$ inhibitors pargyline and pheniprazine fully substituted for cocaine. However, the MAO_A inhibitor clorgyline (also known as clorgiline) and the nonselective MAO inhibitor nialamide did not [27]. Later studies questioned whether deprenyl's substitution for cocaine was actually due to increased levels of monoamines, as deprenyl was shown to produce amphetamine or methamphetamine as a metabolite [28].

Discriminative stimulus effects can be readily established by activating imidazoline I2 receptors, which are located on MAO_A and MAO_B enzymes. The activation of these receptors inhibits both enzymes [29, 30]. Jordan et al. [31] reported that the reversible MAO_A inhibitor moclobemide and the irreversible, nonselective $MAO_{A/B}$ inhibitor pargyline fully substituted for the high affinity imidazoline I2 ligand RX801077. In a later study, MacInnes and Handley [32] found that the selective reversible MAO_A inhibitor RO41-1049 also fully substituted for RX801077, while the reversible MAO_B inhibitors lazabemide and RO16-6491 did not. Thus, the interoceptive stimulus effects produced by I2 receptor-induced inhibition of MAO appear to be more associated with MAO_A inhibition, as compared to MAO_B inhibition.

Downstream receptor actions may mediate the discriminative stimulus effects of MAO inhibitors. Crissman and O'Donnell [33] trained rats to discriminate the β_1-adrenoceptor agonist isoproterenol. Worth noting here is that intracerebroventricular (ICV) administration was used during the initial discrimination training phase for this compound. ICV administration is not commonly used in drug discrimination studies due to the complications of keeping a cannula fixed in place for what are typically lengthy behavioral studies. Even so, once this discrimination was learned, rats were tested with a series of antidepressant drugs for their similarity with isoproterenol. They found that the nonselective $MAO_{A/B}$

Table 1 Receptor mechanisms producing full substitution for antidepressant drugs

Antidepressant type	Mechanisms producing full substitution
MAO inhibitors	Imidazoline I2 agonism β_1-adrenoceptor agonism
Tricyclic antidepressants	β_1-adrenoceptor agonism DA reuptake inhibitor NE reuptake inhibitor 5-HT_{1A} agonism
SSRI	5-HT_{2C} agonism 5-HT_{1A} agonism
SNRI	β_1 receptor agonism
Bupropion (atypical antidepressant)	DA reuptake inhibitor Muscarinic receptor antagonism D_1 receptor agonism D_2 receptor agonism
Reboxetine (atypical antidepressant)	NK_1 receptor antagonism NE reuptake inhibitor

inhibitor phenelzine engendered full substitution for ICV isoproterenol. The authors interpreted these results as being due to enhanced norepinephrine concentrations that activated β_1 receptors.

Overall, we can reach the following general conclusions regarding the discriminative stimulus properties of MAO inhibitors. First, drugs that inhibit MAO_B more selectively than MAO_A produce stimulus effects similar to those produced by certain psychostimulant drugs. However, this conclusion does depend greatly on the particular MAO inhibitor that was tested and the finding that the substitution seen by deprenyl to methamphetamine and cocaine was likely due to deprenyl's active metabolites. This raises the question about the role of other MAO inhibitor active metabolites in the discriminative stimulus effects of these drugs. Second, stimulus effects elicited by directly inhibiting MAO via the imidazoline I2 receptor are similar to those produced by MAO_A inhibitors. Third, enhanced monoamine neurotransmitter concentrations will lead to greater activation of receptors (e.g., β_1 noradrenergic receptors), which may engender properties that add to the overall discriminative stimulus properties of an MAO inhibitor. Table 1 provides a summary of general discriminative stimulus findings for MAO inhibitors and other antidepressant drugs.

3.2 Tricyclic Antidepressant Drugs

As noted earlier, tricyclic antidepressant drugs tend to inhibit norepinephrine and serotonin reuptake, along with exhibiting receptor binding to serotonin, histamine H_1, and muscarinic receptors. In addition to these receptor binding profiles, tricyclic antidepressant drugs also differ in their relative affinity for serotonin versus

norepinephrine transporters. For example, imipramine displays a similar affinity for both serotonin and norepinephrine transporters, while desipramine exhibits over a 20-fold preferential affinity for norepinephrine transporters versus serotonin transporters [7]. In contrast, clomipramine has an approximately 20-fold greater affinity for serotonin transporters than for norepinephrine transporters [14].

Desipramine was the first tricyclic antidepressant drug to be established as a discriminative stimulus. Shearman et al. [34] trained rats to discriminate desipramine from vehicle for food reinforcement using two-lever operant chambers. Neither of the tricyclic antidepressant drugs imipramine or protriptyline substituted for desipramine's discriminative cue; however, amphetamine at a dose of 1.25 mg/kg produced partial substitution (67% drug lever selection) for desipramine. This finding is in agreement with later studies that found that cocaine can produce either full or partial substitution for desipramine [35, 36], although other studies have not [37, 38]. Thus, these drug discrimination studies generally confirm the inhibition of reuptake for serotonin and norepinephrine by desipramine. Other studies have shown that desipramine in combination with a sub-effective dose of cocaine produces full substitution for training doses of cocaine [37, 39]. In the study mentioned earlier by Crissman and O'Donnell [33], desipramine fully substituted for the β_1 receptor agonist isoproterenol, which was likely due to greater activation of these receptors from elevated concentrations of norepinephrine by desipramine.

One of the most studied tricyclic antidepressants in the drug discrimination paradigm is imipramine. As was the case with the MAO inhibitors, Overton [22] first demonstrated that rats could distinguish the stimulus effects of imipramine (40 mg/kg) from a non-drug state using a T-maze drug discrimination procedure in rats. In his study, the stimulus effects were readily discriminated as animals only required approximately 12 sessions to meet the training criterion. Subsequently, Schechter [40] used a two-lever operant chamber to train rats to discriminate imipramine from saline for food reinforcement. Schechter exposed the rats to different types of stressors (e.g., footshock, restraint) in order to produce something more analogous to a "depressive" state in humans. However, only the unstressed rats in this study were successfully trained to discriminate imipramine. Schechter found that the tricyclic antidepressants amitriptyline and desipramine fully substituted for imipramine in this group of rats.

Much of the research on imipramine's discriminative stimulus properties were conducted by Barrett's group in the early 1990s. Zhang and Barrett [41] trained pigeons to discriminate imipramine from vehicle using a two-key operant chamber for food reinforcement. As previously found in rats [40], amitriptyline fully substituted for imipramine. In addition, they reported that the psychostimulant cocaine and the antidepressant drug bupropion (dopamine and norepinephrine transporter inhibitor) also fully substituted for imipramine's discriminative stimulus. The mechanisms shared by imipramine, amitriptyline, and cocaine consist of reuptake inhibition of serotonin and norepinephrine, while imipramine and bupropion share an inhibition of norepinephrine reuptake. Direct receptor actions also might be shared by imipramine and amitriptyline, while enhanced concentrations of serotonin or norepinephrine likely led to downstream receptor actions.

In a follow-up to this study, Barrett and Zhang [42] reported that the 5-HT_{1A} partial agonist 8-OH-DPAT mimicked the discriminative stimulus of imipramine, and that the discriminative stimulus effects of imipramine were blocked by administration of the 5-HT_{1A} receptor antagonist NAN-190. They also found that the α_1 receptor antagonist prazosin blocked the discriminative stimulus effects of imipramine. Imipramine lacks any affinity for 5-HT_{1A} receptors and neither of these drugs appears to exhibit appreciable binding affinities to the same receptors. Imipramine, however, does exhibit a high affinity for α_1 adrenoceptors in humans [6], but only a moderate affinity for α_1 receptors in rats [43] and chronic dosing with imipramine in rats does not produce upregulation of α_1 adrenoceptors [44]. This may account for the ability of prazosin to block the activation of α_1 receptors by imipramine.

Other well-studied tricyclic antidepressants in the drug discrimination literature are clomipramine, nortriptyline, and amitriptyline; however, none of these drugs have been used as the training drug in a discrimination study. As noted earlier in this chapter, Shearman et al. [34] did not find stimulus generalization from desipramine to protriptyline. Further, amitriptyline has been shown to fully substitute for imipramine [40, 41], for the atypical antipsychotic drug clozapine, and for the cholinergic muscarinic receptor antagonist scopolamine in rats [45], likely due to shared stimulus effects mediated by muscarinic receptor antagonism among these compounds. In pigeons, only partial substitution was found reported to clozapine by amitriptyline [46].

3.3 SSRI Antidepressant Drugs

As the primary mechanism of action for SSRIs is an elevation of extracellular serotonin concentrations due to inhibition of reuptake, it is worth considering the discriminative stimulus properties of "serotonin releasers" — drugs that elevate serotonin concentrations by a variety of different mechanisms but do not produce antidepressant effects. One of the primary serotonin releasers used in behavioral pharmacology is fenfluramine. Fenfluramine acts as an effective anorectic in humans (although it has serious side effects), which is likely due to activation of 5-HT_{2C} receptors via elevated serotonin concentrations [47]. Fenfluramine increases extracellular serotonin release through an exocytosis-like mechanism at lower concentrations and by reversal of the serotonin transporter at higher concentrations [48], but does not bind to serotonin receptors. Goudie [49] established fenfluramine as a discriminative stimulus, using female rats in a two-lever operant task for food reinforcement, finding partial substitution of fenfluramine's metabolite norfenfluramine, which is now known to activate both 5-HT_{2B} and 5-HT_{2C} receptors [47] and produce greater increases in norepinephrine concentrations than fenfluramine [50]. Subsequent drug discrimination studies found full substitution by serotonin/norepinephrine releasers [51, 52], the serotonin receptor agonist quipazine (which exhibits a high affinity for 5-HT_3 and a moderate affinity for 5-HT_{2B} and 5-HT_{2C} receptors) [52], the 5-$HT_{2C/1B}$ receptor agonist *m*-

chlorophenylpiperazine (mCPP), and the selective 5-HT_{2C} receptor agonist MK-212 [53]. Partial stimulus generalization occurred to the 5-HT_{2A} preferring (over 5-HT_{2C}) receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI), and full blockade of the fenfluramine discriminative stimulus occurred following pretreatment with the 5-HT_{2C} receptor antagonist SB206553, but not the 5-HT_{2C} receptor antagonist RS102221 [53]. Thus, it appears that activation of 5-HT_{2C} receptors may yield stimulus properties more similar to the discriminative stimulus properties of fenfluramine than does activation of other serotonin receptor subtypes. The prominence of 5-HT_{2C} mediated properties of the fenfluramine discriminative stimulus provides a compelling link to the primary receptor likely mediating fenfluramine's anorectic effects. Further, these findings offer some predictive value for the discriminative stimulus properties of SSRIs.

There are several difficulties to consider when establishing the discriminative stimulus properties of SSRIs. One of the first published attempts to establish fluoxetine as a training drug was reported by Marona-Lewicka and Nichols [54]. While this study successfully established the SSRIs citalopram and sertraline as discriminative stimuli in male rats, subjects could not reliably discriminate fluoxetine from vehicle after a year of training. The authors suggested that the long half-life of fluoxetine, which is close to a week in humans, precluded discriminating fluoxetine versus a true non-drug interoceptive state. Thus, fluoxetine is not an ideal candidate drug for discrimination training, but other SSRIs with half-lives of approximately 24 h or less in human – including citalopram, fluvoxamine, paroxetine, or sertraline – might be more easily established as discriminative stimuli [55].

Millan et al. [56] also established citalopram as a discriminative stimulus in rats after first determining doses of citalopram that elevate extracellular serotonin concentrations. Male rats successfully learned to discriminate citalopram (2.5 mg/kg) from vehicle in a two-choice procedure using food reinforcement. During generalization testing, both of the SSRIs, paroxetine and sertraline, fully substituted for citalopram. In a second study, Millan et al. [57] reported full stimulus generalization to the 5-HT_{2C} receptor agonist RO60-0175 and full stimulus blockade by the 5-HT_{2C} receptor antagonist SB242,084. Thus, like fenfluramine, 5-HT_{2C} receptor activation appears to mediate the discriminative stimulus properties of citalopram (also see review by [58]). Yet, unlike fenfluramine, citalopram, and indeed many other SSRIs with the exception of fluoxetine, causes weight gain in clinically depressed patients. In fact, weight gain is often attributed to SSRI treatment regimens [59].

Wolff and Leander [60] established the putative SSRI antidepressant, LY233708, as a discriminative stimulus in pigeons. LY233708 exhibited a relatively short half-life and serotonin concentrations returned to baseline levels a day after administration. Full stimulus generalization occurred to fluoxetine and citalopram, but not to the norepinephrine reuptake inhibitor nisoxetine. Full stimulus generalization also occurred from the 5-HT_{1A} receptor agonist 8-OH-DPAT, and pretreatment with the 5-HT_{1A} receptor antagonist WAY100,635 fully blocked the LY233708 discriminative cue.

Conditioned taste aversion (CTA) is a procedure with similar behavioral endpoints as classical drug discrimination assessments and has been useful for assessing the discriminative stimulus effects of SSRIs. As described by Riley and colleagues in Chap. 14 of this volume, a typical CTA drug discrimination paradigm utilizes the novelty of a drug effect paired with an unfamiliar (normally sweet-tasting) substance to reduce drinking of the unfamiliar substance during a subsequent test session. Yet, if the same or a similar drug were given during the preceding sessions that contained only tap water in the bottles (referred to as training sessions), then the drug given on the conditioning session would ineffectively pair with the novel tasting solution and lead to significant consumption of the solution on the test day.

Using the CTA drug discrimination procedure, Berendsen and Broekkamp [61] examined the discriminative stimulus effects of fluoxetine in male mice. Without "pre-exposure" drug administration (i.e., drugs given during training sessions), fluoxetine administration on the conditioning day led to significantly less consumption of the glucose solution during the test session. However, using fluoxetine as both a pre-exposure drug and a test drug led to increased consumption of the solution on the test day. A similar effect occurred when the 5-HT_{2C} receptor agonist MK212 was used as a pre-exposure drug, and fluoxetine as a test drug, demonstrating that fluoxetine and MK212 exhibited similar stimulus effects. This did not occur when the 5-$HT_{2A/2C}$ receptor agonist DOI was used as the pre-exposure drug. A partial reduction of glucose solution intake was observed when the 5-HT_{1A} receptor partial agonist 8-OH-DPAT was used as the pre-exposure drug. Thus, these results tend to support the involvement of 5-HT_{2C} receptors, and to some extent 5-HT_{1A} receptors, mediating the discriminative stimulus effects of fluoxetine. Other SSRIs also have been studied using the CTA drug discrimination paradigm. For example, Gommans et al. [62] found that the SSRI fluvoxamine shared similar stimulus effects with 5-HT_{1A} receptor partial agonists, but not to 5-HT_{2A} or 5-HT_{2C} receptor agonists or to the SSRI fluoxetine in this study.

Overall, drug discrimination studies demonstrate that serotonin releasers (which do not appear to exhibit antidepressant effects) and SSRIs with clinical antidepressant efficacy can both be established as discriminative stimuli. However, operant drug discrimination procedures normally require daily training sessions – an obstacle when using compounds with long half-lives as training drugs. The CTA drug discrimination procedure appears to obviate this limitation, as this procedure allows for fluoxetine and other SSRIs to be established as discriminative stimuli. From serotonin releaser and SSRI drug discrimination studies, we learn that these compounds tend to exhibit generalization to each other, with occasional exceptions (e.g., [62]). In conclusion, the discriminative stimulus effects of SSRIs appear to be mediated primarily by activation of 5-HT_{2C} receptors and to a lesser extent by 5-HT_{1A} receptors, and probably not at all by 5-HT_{2A} receptors.

3.4 SNRI Antidepressant Drugs

The only study establishing an SNRI as a discriminative stimulus was conducted by Kayir et al. [63]. Using the CTA drug discrimination procedure, this group found that drugs that increased both serotonin (e.g., fluoxetine) and norepinephrine reuptake inhibition (e.g., reboxetine) fully substituted for the SNRI venlafaxine. Several studies have examined the stimulus effects of SNRIs in drug discrimination studies using these compounds as test agents rather than as training drugs. These studies generally support the idea that the stimulus properties of venlafaxine are elicited by enhanced serotonin and norepinephrine efflux. For example, venlafaxine has been shown to potentiate the discriminative stimulus effects of the hallucinogen LSD [64] and to fully substitute for the β_1 receptor agonist isoproterenol [33]. Similarly, the SNRI sibutramine has been shown to substitute for the psychostimulant cocaine [65].

3.5 Atypical Antidepressant Drugs

As noted earlier, atypical antidepressant drugs include those that do not fit the previously mentioned categories. While the best known drugs in this class are reboxetine (a selective norepinephrine reuptake inhibitor) and bupropion (a selective dopamine reuptake inhibitor), the first selective norepinephrine reuptake inhibitor to be studied using drug discrimination was the + enantiomer of oxaprotiline (tetracyclic family, related to the antidepressant maprotiline). Filip et al. [66] trained male rats to discriminate (+)-oxaprotiline from vehicle, finding full stimulus generalization to the tricyclic antidepressant drug desipramine.

Using a two-lever discrimination for food reinforcement in rats, Dekeyne et al. [67] first established reboxetine as a discriminative stimulus and used in vivo microdialysis to measure extracellular monoamine neurotransmitter concentrations to verify significant elevations of norepinephrine, but not serotonin, in the frontal cortex and hippocampus. Full stimulus generalization occurred from reboxetine to the SNRI venlafaxine and to antidepressants that preferentially block norepinephrine reuptake, desipramine, and maprotiline. Stimulus generalization did not occur to drugs lacking this mechanism. Millan and Dekeyne [68] later reported on an extensive evaluation of the discriminative stimulus properties of reboxetine, finding full stimulus generalization to all compounds that produced increases in norepinephrine concentrations via inhibition of norepinephrine reuptake. No appreciable degree of stimulus generalization occurred to SSRIs lacking an affinity for norepinephrine transporters. Stimulus generalization also did not occur to adrenoceptor agonists, although α_1 adrenoceptor antagonists fully, and an α_2 receptor antagonist partially, blocked the discriminative cue. Also, the NK1 receptor antagonist GR205,171 fully substituted for reboxetine. It is thought that NK1

receptor antagonists may elevate norepinephrine concentrations given that increased norepinephrine concentrations are observed in $NK1^{-/-}$ mice [68].

Howard and colleagues ([69]; portions of this study were reported previously in a book chapter, [70]) were the first to determine that the selective dopamine reuptake inhibitor bupropion could serve as a discriminative stimulus. In this study, bupropion's discriminative stimulus generalized to psychostimulants (d-amphetamine, cocaine, benzylpiperazine, methylphenidate, and caffeine) and to some antidepressant drugs (nortriptyline, viloxazine, and nomifensine), but not others (imipramine, amitriptyline, desipramine, and mianserin). Full stimulus generalization also occurred to the muscarinic receptor antagonist scopolamine, despite bupropion having no affinity for muscarinic receptors [71]. Interestingly, scopolamine also has been found to substitute for the tetracyclic antidepressant mianserin, which has minimal affinity for cholinergic receptors, although mianserin did not substitute for scopolamine [72]. Later, Blitzer and Becker [73] also reported full stimulus generalization to the psychostimulants amphetamine, cocaine, and caffeine, but were unable to antagonize bupropion's discriminative cue by antipsychotic drugs like haloperidol and thioridazine, which are potent D_2 receptor antagonists. Based on a lack of blockage by D_2 receptor antagonists, the authors concluded that bupropion's discriminative cue was not mediated by dopaminergic mechanisms, nor was it mimicked or blocked by adrenergic or serotonergic drugs.

However, some clarification of the discriminative stimulus properties of bupropion was made as more selective dopaminergic compounds became available [74]. In addition to verifying that the psychostimulant cocaine produced full stimulus generalization from bupropion, this study found that dopamine reuptake blockers fully substituted for bupropion, that full or partial substitution was observed for a series of dopamine D_1 and D_2 receptor agonists and that D_1 and D_2 receptor antagonists partially blocked the bupropion cue. Based on these results the authors concluded that the discriminative stimulus effects of bupropion are mediated by activation of D_1 and D_2 receptors. The finding that scopolamine also mimics bupropion's discriminative cue [71] warrants further investigation as it is possible that muscarinic mechanisms may play an indirect role in bupropion's discriminative stimulus properties and perhaps even in its antidepressant efficacy (see section below on use of scopolamine for the treatment of depression).

4 Discriminative Stimulus Properties of Anxiolytic drugs

Although various remedies for nervousness and anxiety were used for many years, including opiates, bromide salts, and alcohol, we recognize the discovery of barbiturates, the so-called "minor tranquilizers," as the first anxiolytic medications (antipsychotic drugs were called "major tranquilizers"). The first barbiturate effective in humans, barbital (Veronal®), was brought to market in 1904 and many of the other 49 barbiturates came soon after. While effective for anxiety, their propensity

Table 2 Receptor mechanisms producing full substitution for anxiolytic drugs

Anxiolytic class	Mechanisms producing full substitution
Barbiturates	$GABA_A$ receptor positive modulators
Benzodiazepines	$GABA_A$ receptor positive modulators BZ I agonism BZ II agonism
Buspirone	5-HT_{1A} agonism

for causing dependence and risk of lethal overdose led to serious public health concerns [75, 76].

Both barbiturates and benzodiazepines bind to allosteric sites near the Cl^- channel for $GABA_A$ receptors. Activation of these sites modulates the receptor to be more responsive to activation of the $GABA_A$ receptor site (i.e., positive modulation). Benzodiazepines have two sites: BZ I and BZ II. The BZ I type is found on the α_1 subunit containing the $GABA_A$ receptor binding site [77], while the BZ II site is found on α_2, α_3, and α_5 subunits [78, 79]. BZ I $GABA_A$ receptors are found in the thalamus, substantia nigra, and cerebellum, while BZ II $GABA_A$ receptors are found in cerebral cortex, hypothalamus, and amygdala [80]. The sleep aids zolpidem and eszopiclone show preferential binding to the BZ I site versus the BZ II site [81], which accounts for their sedative effects while lacking anxiolytic effects [82, 83].

Aside from drugs acting at $GABA_A$ receptors, the 5-HT_{1A} receptor agonist buspirone (BuSpar®) also is prescribed as an anxiolytic drug. Buspirone appears to be as effective as benzodiazepines for treating anxiety disorders [84], but treatment effects for anxiety do not appear until after 4–6 weeks of treatment. The delayed treatment response appears to be why many practitioners are skeptical about its effectiveness as an anxiolytic [85].

Today, first-line treatments for anxiety consist primarily of antidepressant drugs that inhibit serotonin reuptake. These agents avoid abuse concerns over benzodiazepine use and offer prophylactic effects toward avoiding anxiety episodes. Yet, we still refer to these drugs as antidepressant drugs, rather than anxiolytic drugs, to address their primary clinical use. Patients also may need to take benzodiazepines during antidepressant treatment on an "as needed" basis for addressing acute episodes of anxiety, such as a panic attack [86]. Drug discrimination studies involving antidepressant drugs were reviewed in Sect. 3 and this section will address the discriminative stimulus properties of barbiturate, benzodiazepine, and 5-HT_{1A} receptor agonist drug classes. Table 2 shows a general summary of the discriminative stimulus properties for anxiolytic drugs.

4.1 Barbiturates

Most of what we know about the discriminative stimulus properties of barbiturates were sorted out in the 1970s and early 1980s. Hirschhorn and Winter [87] first

established a barbiturate as a discriminative stimulus by training rats to discriminate barbital from saline. This study found that its stimulus properties were not similar to those produced by 5-HT_2 receptor agonists including the hallucinogens mescaline or LSD. York [88] later reported that male rats trained to discriminate either barbital or phenobarbital from saline did not evoke either partial or full generalization to ethanol. In male pigeons, Herling et al. [89] found full stimulus generalization occurring from pentobarbital to other barbiturates as well as to benzodiazepines. The CNS stimulant and pro-convulsant bemegride blocked discriminative control by pentobarbital, and stimulus generalization did not occur from pentobarbital to opioid agonists or anticonvulsants. Stimulus generalization also did not occur to the $GABA_A$ agonist and $GABA_A$-rho partial agonist muscimol or to the $GABA_B$ receptor agonist baclofen. Similar findings were shown in male rhesus monkeys trained to discriminate pentobarbital from vehicle [90]. Non-rate suppressant doses of the benzodiazepine antagonist Ro 15-4513 did not block the discriminative stimulus effects of phenobarbital in male mice [91].

Barbiturates elicit discriminative stimulus effects that are most similar to those generated by other barbiturates, but benzodiazepines elicit surprisingly similar interoceptive effects. These stimulus effects likely occur at doses capable of engendering anxiolytic effects, as pentobarbital has been shown to increase rates of punished responding in a drug discrimination procedure in pigeons [92]. Licata et al. [93] provided a refinement on our understanding of the stimulus effects produced by barbiturates by showing that the $GABA_A$ receptor positive modulator L-838,417, which is selective for alpha 2, 3, and 5 subunits (those corresponding with the BZ II site), fully substituted for the barbiturates amobarbital and pentobarbital in squirrel monkeys.

4.2 Benzodiazepines

Not surprisingly, the discriminative stimulus properties of benzodiazepines appear to be mediated primarily by benzodiazepine receptors, and their discriminative stimulus properties also are similar to stimulus effects produced by other $GABA_A$ positive modulators. Colpaert et al. [94] first evaluated the discriminative stimulus effects of a benzodiazepine by training male rats to discriminate chlordiazepoxide from vehicle in a two-lever operant task. Full stimulus generalization occurred to all of the benzodiazepines tested and to all of the barbiturates tested. Neither partial nor full generalization occurred to the $GABA_B$ agonist baclofen. Haug [95] found that the discriminative stimulus effects of diazepam were completely blocked by the convulsant pentylenetetrazol (PTZ), which binds to the picrotoxin site within the $GABA_A$ channel, and partially blocked by the $GABA_A$ orthosteric antagonist bicuculline. Young and Glennon [96] found full stimulus generalization from diazepam to at least 17 other benzodiazepines. This later study found a nearly perfect positive correlation between each benzodiazepine's ED_{50} dose for diazepam-lever responding and the lowest effective therapeutic dose in humans.

Woudenberg and Slangen [97] found that midazolam fully generalized to other benzodiazepines tested, but also generalization did not occur to buspirone. Benzodiazepines have been evaluated as discriminative stimuli in humans too, as reviewed in Chap. 13 of this volume. For example, Johanson found that humans could discriminate diazepam from placebo and that the discriminative stimulus effects of diazepam were similar to those of lorazepam [98] and triazolam [99], but not to those produced by buspirone [99].

The imidazopyridine hypnotic zolpidem, which is a nonbenzodiazepine, binds preferentially to BZ I sites and does not substitute for chlordiazepoxide in male rats [100]. In baboons, however, full stimulus generalization occurs from lorazepam or from the barbiturate pentobarbital to zolpidem [101]. In male rats, full generalization occurred from zolpidem's discriminative stimulus to the BZ I preferring agonist zopiclone [81], to benzodiazepines, and to a barbiturate.

Mintzer et al. [102] demonstrated that the discriminative stimulus effects between zolpidem and the benzodiazepine triazolam can be differentiated by using a three-choice drug discrimination procedure in human subjects. Such a discrimination might be expected to focus on BZ II-elicited stimulus properties by the benzodiazepine and the BZ I-elicited stimulus properties by zolpidem. Yet, when asked to fill out subjective effect questionnaires for comparing zolpidem to triazolam, participants endorsed terms such as "blurred vision," "dry mouth," and "nervous" to the zolpidem condition and the majority of the remaining terms endorsed were equivalent between these two drugs. However, a number of subjective effect terms on the questionnaires differentiated these drugs from placebo. Rush et al. [103] confirmed that humans can discriminate zolpidem from placebo using a two-choice drug discrimination procedure and that full stimulus generalization occurs to benzodiazepines and barbiturates.

One of the aims of current benzodiazepine drug discrimination research is to assess stimulus effects for particular alpha subtypes of the $GABA_A$ receptor. Licata et al. [93] used a highly selective BZ II agonist, L-838,417, as a training drug in male squirrel monkeys, finding full stimulus generalization to benzodiazepines and barbiturates, and also to the BZ I preferring agonists zolpidem or zopiclone. Further research in this area will require more selective BZ I receptor ligands.

4.3 Buspirone

Buspirone functions as an agonist at serotonin 5-HT_{1A} receptors, with a binding affinity of approximately 20 nM [104]. Buspirone does bind with a moderate to weak affinity for 5-HT_6 (~400 nM) [105] and 5-HT_7 (376 nM) [106] receptors and has a somewhat stronger affinity (78.0 nM) for dopamine D_4 receptors [107]. Hendry et al. [108] first discovered that buspirone, as the training drug, elicits stimulus effects that differ from those of benzodiazepines and barbiturates. A later study confirmed that buspirone did not share discriminative stimulus properties with benzodiazepines in baboons and rats [109]. Moreover, humans can

differentiate the discriminative stimulus effects of acute buspirone versus diazepam and placebo in a three-choice procedure [110].

Stimulus effects elicited by 5-HT_{1A} receptor agonism are highly relevant to the buspirone cue, as buspirone fully substitutes in rats [111] and in pigeons [112] that were trained to discriminate the 5-HT_{1A} agonist 8-OH-DPAT. This substitution is symmetrical as 8-OH-DPAT fully substitutes for buspirone in pigeons [113]. When drugs cross-generalize to each other, this is usually a strong indication of similar underlying mechanisms mediating their discriminative stimulus properties [114]. Sanger [115] did report full stimulus generalization occurring from the α_2 adrenoceptor antagonist idazoxan to buspirone in male rats, but as other 5-HT_{1A} receptor agonists fully substitute for idazoxan [115, 116], the shared discriminative stimulus properties between buspirone and idazoxan are likely mediated by activity at 5-HT_{1A} receptors.

There is some evidence for a dopaminergic component partially mediating the buspirone discriminative cue. Buspirone has been shown to fully block an apomorphine discriminative stimulus in squirrel monkeys (two males, one female) [117] and d-amphetamine in rhesus monkeys (two males, two females) [118]. Further, buspirone partially attenuates a cocaine discriminative cue in male rats [119] and partially generalizes to the D_2 preferring antagonist haloperidol [120]. Rijnders and Slangen [121] reported similar findings, showing partial generalization from buspirone to haloperidol, sulpiride, and the D_2 receptor antagonist R 79598.

5 Discriminative Stimulus Properties of Novel Therapeutic Targets for the Treatment of Depression

While the monoamine hypothesis has been firmly anchored for over 50 years as the predominate theory explaining the underlying neuropharmacology of depression (see reviews by [4, 5]), there has been a great deal of interest in recent years in targeting other, non-aminergic mechanisms as novel therapeutic targets for the treatment of depression [122]. In fact, cholinergic [123, 124] and glutamatergic [4, 5] processes are being considered as novel antidepressant treatment strategies. While the drug discrimination literature has historically focused on the abuse liability of cholinergic- and glutamatergic-acting drugs, there are emerging experimental programs devoted to relating cholinergic and glutamatergic compounds with antidepressant mechanisms of action. We will address each of these areas below. Table 3 provides a summary of these findings.

Table 3 Novel antidepressant strategies

Pharmacological mechanism	Rationale	Stimulus substitution findings
Anticholinergic (muscarinic)	Clinical data showing improvements in unipolar and bipolar depression Rapid antidepressant effects May specifically involve muscarinic M_2 receptors	Muscarinic receptor antagonism in CNS
Glutamatergic NMDA receptor antagonism	Clinical data showing improvements in unipolar and bipolar depression Rapid antidepressant effects Long-lasting antidepressant effects	No substitution by a glycine site partial agonist

5.1 Anticholinergic Drugs as Antidepressants

A number of studies have touted the possible antidepressant effects of anticholinergic drugs. As early as 1981, the anticholinergic drug biperiden (Akineton®) was administered to patients with major depressive disorder [125]. That study reported a significant improvement in symptoms, but had to be discontinued after 3 weeks in two patients due to side effects. However, a later study using a double-blind procedure failed to find a reliable antidepressant effect for biperiden [126]. More promising results have been reported for the anticholinergic scopolamine. A recent systematic review of the literature by Jaffe et al. [127] found seven studies that evaluated mood and depression after a low dose of intravenous scopolamine administration (ranging from 3 to 5 days of administration, either on consecutive or intermittent days). Based on their review of the available literature, they concluded that "Scopolamine is an effective and rapid antidepressant in both unipolar and bipolar depression. . .." with patients exhibiting significant reductions in depressive symptoms with 3 days after the first administration of scopolamine. They noted that no patients dropped out of the studies due to secondary effects, although subjective confusion was typically reported by patients 2 h after infusion of scopolamine. In a placebo-controlled clinical trial scopolamine induced drowsiness, blurred vision, dry mouth, light-headedness, and reduced blood pressure, but these side effects were well tolerated and no subjects dropped out of the clinical trial. Scopolamine infusions produced a rapid and robust antidepressant response in the patients. Je Jeon et al. [124] examined the role of muscarinic receptors in the pathophysiology of mood disorders and concluded that a body of recent evidence supports the role of muscarinic cholinergic receptors in the pathophysiology of both major depressive disorders and bipolar disorders. Specifically, they argue that targeting the cholinergic M_2 receptors might produce more rapid and robust clinical effects, especially if new drugs could be developed that have minimal or no peripheral cholinergic effects (see also [18, 128, 148]). Clearly, drug discrimination studies could play an important role in delineating the receptor pharmacology of novel therapeutic compounds. While the drug discrimination research on anticholinergic drugs has not focused on this particular topic, there have been a number of

studies that have examined the discriminative stimulus properties of anticholinergic drugs and a few that lend some support to the idea of therapeutic properties for anticholinergic drugs.

The cholinergic antagonist atropine was one of the first drugs studied in a two-lever drug discrimination assay and, as would be expected, scopolamine fully substituted for atropine's discriminative stimulus [129]. Interestingly, they also demonstrated that atropine's discriminative cue was centrally mediated as atropine methyl bromide, which is a quaternary compound that does not readily cross the blood–brain barrier, and did not produce any atropine-appropriate responding. Later, Jung et al. [130] examined the discriminative stimulus properties of scopolamine as the training drug. He reported similar results about scopolamine's discriminate cue being centrally mediated (scopolamine methylbromide, which does not readily cross the blood–brain barrier, did not substitute for scopolamine) and reported that scopolamine's cue was mediated by antagonism of muscarinic receptors. This finding was not surprising given scopolamine's very potent and selective binding at all five (M_1–M_5) muscarinic cholinergic receptors (<2.1 nM, obtained from PDSD database on 20 March 2016). Unfortunately, there has been no systematic examination of the ability of various antidepressant drugs to mimic scopolamine's discriminative cue. However, scopolamine has been a test drug in other studies in which antidepressants or antipsychotic drugs have been established as the training drug. For example, Jones et al. [69] established the atypical antidepressant bupropion (see earlier discussion in this chapter about bupropion) as a discriminative stimulus in a two-lever discrimination procedure in rats. They found that a dose of 0.5 mg/kg scopolamine produced full substitution for bupropion in 2 of 6 rats (responding was significantly diminished in the other 4), albeit no reliable substitution was evident at doses above and below 0.5 mg/kg. This variability in results for scopolamine was attributed to disruptions in response rates. These findings suggest that scopolamine might possibly share some discriminative stimulus properties with bupropion, but response disruption obviously prevents any definite conclusions. One approach would be to use a low, non-generalizing dose of bupropion in combination with low, non-generalizing doses of scopolamine; if these compounds share discriminative stimulus properties, then a greater level of stimulus generalization would be expected. More research in this area is needed to test this hypothesis.

Kelley et al. [72] established a two-lever drug discrimination with the tetracyclic antidepressant mianserin (4.0 mg/kg) in one group of rats and scopolamine (0.25 mg/kg) in another. An asymmetrical generalization between mianserin and scopolamine was observed. Scopolamine produced mianserin-appropriate responding in the mianserin-trained rats, but mianserin did not produce scopolamine-appropriate responding in the scopolamine-trained rats. While the underlying mechanisms responsible for mianserin's discriminative stimulus properties have not been delineated, it is based at least in part by antagonism of certain serotonergic receptors [131–133]. In particular, mianserin exhibits a high affinity for serotonin 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors [134]. However, given that the very selective muscarinic cholinergic antagonist scopolamine fully substituted for

mianserin, there does appear to be a cholinergic component to mianserin's discriminative cue.

5.2 *Glutamatergic Drugs as Antidepressants*

Since Berman and colleagues conducted a proof of concept study in 2000 demonstrating that low dose intravenous ketamine could produce both rapid and sustained antidepressant effects, there has been a surge of recent interest into examining glutamatergic drugs as potentially novel therapeutic targets for the treatment of depression. While a lot of this research has focused on the therapeutic use of ketamine in the clinic, a large number of studies have been examining the underlying glutamatergic mechanisms that are responsible for ketamine's antidepressant effects [19]. There are a number of more recent studies that have examined glutamatergic mechanisms as targets for antidepressant effects, and several are currently in clinical trials (see reviews by [5], [135]). As was the case with scopolamine and other anticholinergic drugs, the drug discrimination literature on ketamine and other noncompetitive NMDA antagonists is considerably lacking, having focused more on the abuse liability of these drugs rather than their potential for having therapeutic effects. For example, GLYX-13 (Rapastinel®) is a "functional" partial agonist at the NMDA receptor glycine site (it does not bind directly to the glycine site) currently in phase III clinical trials for use as an adjunctive therapy in treatment-resistant major depressive disorder. In a recent study rats were trained to discriminate 10 mg/kg ketamine from saline vehicle [136]. Then varying doses of GLYX-13 were tested for generalization to ketamine. In doses up to 156 mg/kg, GLYX-13 did not generate ketamine-associated responding (i.e., it did not substitute for ketamine) and did not produce a suppression of response rates. Based on these results, the authors concluded that Glyx-13 did not share discriminative stimulus properties with ketamine and exhibited no sedative or abuse-related side effects. These results profile a preliminary, yet highly promising, role for glutamatergic-acting agents with highly selective antidepressant effects.

To the best of our knowledge, there have been no other published studies in which ketamine was the discriminative stimulus and current (or potential) antidepressant or anxiolytic drugs have been tested for generalization. This clearly represents a future area of research that is needed. Drug discrimination studies could also play a valuable role in helping to delineate the possible role of mechanisms (either direct or indirect) that mediate ketamine's antidepressant effects at the NMDA receptor.

6 Discriminative Stimulus Properties of Adjunctive Strategies for Antidepressant Drugs

The atypical antipsychotics aripiprazole (Abilify®) and quetiapine (Seroquel®) are used as adjunctive treatments for depressive disorders [137], and quetiapine is often used as a first-line treatment for bipolar disorder [86, 138, 139] and its antidepressant effects may be due in part to its active metabolite *N*-desalkylquetiapine (norquetiapine) (see [140, 141]). Jensen et al. [141] have shown that *N*-desalkylquetiapine has its highest binding affinity at histamine H_1 receptors and displays a moderate affinity at the norepinephrine reuptake transporter, serotonin receptors, the α_{1B} adrenoceptor, and muscarinic receptors (M_1, M_3, and M_5). While it is not known which of these receptor mechanisms may play a role in quetiapine's antidepressant effects, the discriminative stimulus properties of quetiapine have been examined by Goudie and colleagues. The atypical antipsychotics clozapine, olanzapine, and risperidone fully substituted for quetiapine, but the typical antipsychotics haloperidol, chlorpromazine, and loxapine, and the atypical antipsychotic amisulpride did not [142]. In a subsequent study the underlying receptor mechanisms for quetiapine's discriminative cue were examined [143]. The only selective ligand that fully substituted for quetiapine was the muscarinic antagonist, scopolamine (87% drug lever responding), but partial substitution was seen with the α_1-adrenoceptor antagonist prazosin, the presumed preferential dopamine D_3 receptor antagonist PNU 91194A, and the 5-$HT_{2A/2B/2C}$/H_1/M_{1-5} antagonist cyproheptadine. Based on these results, Goudie et al. concluded that quetiapine's discriminative stimulus properties reflect a "compound" cue involving several receptors, but clearly, muscarinic antagonism was sufficient, although not necessary, to mimic quetiapine's discriminative cue. Given that the parent drug quetiapine has very low affinity for muscarinic receptors, this result can probably be attributed to the muscarinic antagonism exhibited by quetiapine's metabolite *N*-desalkylquetiapine [141]. In addition to *N*-desalkylquetiapine, active metabolites for a number of other drugs also have potential as antidepressant drugs [144]. Thus, the drug discrimination assay can be used to help elucidate the receptor mechanisms of these (and other) drugs and to help in the development of future, novel medications for the treatment of depression.

7 Conclusion

The drug discrimination procedure has been extensively used to evaluate the stimulus properties of antidepressant and anxiolytic drugs. We find drug discriminative data on all classes of antidepressants and anxiolytics, including non-anxiolytic hypnotics that bind to benzodiazepine receptors. Moreover, drugs from most of these different classes have been evaluated in humans, lending an assessment of the translational value of these procedures. In general, the stimulus

properties of drugs shown in animals tend to be displayed in humans. While attempts have been made in humans to qualitatively identify the characteristics of the discriminative stimulus effects of drugs, the most reliable predictor of a drug's discriminability remains its action at the receptor level.

A potential shortcoming of these drug discrimination studies, and a shortcoming that is shared by the vast majority of behavioral pharmacology studies, is the use of only male subjects. From the few studies included in this chapter that included both male and female subjects, there does not appear to be differences in the stimulus of the drugs tested. Yet, given that depression and anxiety are more prevalent in women than men [145], much may be gained by learning more about potential sex differences in the stimulus properties of anxiolytic and antidepressant drugs.

Finally, it is worth noting that all of the current prescribed antidepressant drugs and the anxiolytic drug buspirone require weeks of chronic administration in order for therapeutic efficacy to occur. While drug discrimination training does indeed require weeks to meet a high accuracy criterion, the intermittent nature of treatment and placebo days: (1) may not be sufficient for a drug to produce drug effects unique to *chronic* administration, and (2) may engender altered sensitivity to some of the behavioral effects and interoceptive effects of compounds commonly used to treat affective disorders [146]. In fact, it is necessary for a drug's effects to be absent during vehicle test sessions, as demonstrated by a failed to attempt to establish fluoxetine as a discriminative stimulus. For example, drug discrimination studies with antidepressants and buspirone likely only represent acute activity at CNS receptors. Future drug discrimination studies with antidepressants utilizing a procedure that involves a chronic dosing regimen would provide much needed predictive and face validity to the preclinical literature. Many behavioral probes of affective responses are advantageous with regard to their swift and high throughput nature [147]. Clearly, the overwhelming strength of using conditioning procedures for assessing affective measures of drug action, as with drug discrimination, stems from their durability and reproducibility.

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Pharmacokinetic–Pharmacodynamic (PKPD) Analysis with Drug Discrimination

S. Stevens Negus and Matthew L. Banks

Abstract Discriminative stimulus and other drug effects are determined by the concentration of drug at its target receptor and by the pharmacodynamic consequences of drug-receptor interaction. For in vivo procedures such as drug discrimination, drug concentration at receptors in a given anatomical location (e.g., the brain) is determined both by the dose of drug administered and by pharmacokinetic processes of absorption, distribution, metabolism, and excretion that deliver drug to and from that anatomical location. Drug discrimination data are often analyzed by strategies of dose-effect analysis to determine parameters such as potency and efficacy. Pharmacokinetic–Pharmacodynamic (PKPD) analysis is an alternative to conventional dose-effect analysis, and it relates drug effects to a measure of drug concentration in a body compartment (e.g., venous blood) rather than to drug dose. PKPD analysis can yield insights on pharmacokinetic and pharmacodynamic determinants of drug action. PKPD analysis can also facilitate translational research by identifying species differences in pharmacokinetics and providing a basis for integrating these differences into interpretation of drug effects. Examples are discussed here to illustrate the application of PKPD analysis to the evaluation of drug effects in rhesus monkeys trained to discriminate cocaine from saline.

Keywords Acute tolerance • Cocaine • Drug discrimination • Hysteresis • Pharmacodynamics • Pharmacokinetics • Prodrug

S.S. Negus (✉) and M.L. Banks
Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, VA 23298, USA
e-mail: sidney.negus@vcuhealth.org

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Contents

1 Introduction

Drugs produce their effects by interacting with receptor targets, and drug discrimination is one behavioral procedure that is useful for investigating determinants of this interaction. In conceptualizing drug-receptor interactions in whole organisms, it is convenient to think of the receptors as relatively fixed in anatomical space, whereas each dug molecule embarks on a journey from its site of administration, through the body to the receptor upon which it acts, and then back out of the body. Pharmacokinetics and pharmacodynamics are subdisciplines within the field of pharmacology that address two facets of this journey. Pharmacokinetics (PK) is concerned with the processes that govern a drug's path through the body and its resulting concentration in different body compartments. Pharmacodynamics (PD), in contrast, is concerned with the physiological and behavioral consequences produced by that subset of drug molecules that find and occupy receptors during their journey through the body.

The relationship between PK and PD is described by PKPD analysis that relates drug concentration to drug effect. This type of analysis provides an alternative to conventional "dose-effect" analysis of drug effects, and they have value for at least three reasons [1]. First, drug effects are ultimately determined by drug concentration at the receptors upon which the drug acts, and that concentration is determined not only by the drug dose administered, but also by the PK processes that deliver that dose to and from the receptors. "Dose" is a measure of the amount of drug determined prior to its delivery, often in units of drug mass relative to the mass of the organism (e.g., mg/kg). Dose is precisely controlled by the experimenter, and it often serves as the principal independent variable in analysis of data from in vivo studies. For example, the "dose-effect curve" is a common mode of data presentation used to estimate critical drug features such as potency and efficacy. However, after a dose is administered, the drug must be absorbed into the body from the site of its administration (e.g., absorbed from gastrointestinal tract into the blood stream after oral delivery) and distributed from that site to the sites where receptors are located (e.g., distributed by the circulatory system from the gastrointestinal tract to

brain). Moreover, drug molecules are subject to degradation via metabolism by enzymes and to removal from the body via excretion by routes such as urine, feces, or exhaled air. Together, these PK processes of absorption, distribution, metabolism, and excretion convert a drug dose administered at a single anatomical site and a single point in time into a dynamic tide of drug concentrations that rises and then falls throughout the body over time. These changing drug concentrations through time can then be related to changing drug effects through time to yield a richer data set than can be achieved by a reference to only a single drug dose administered at the beginning of an experiment. The most precise assessment of this relationship between drug concentration and drug effect would ideally measure drug concentrations at the site of receptors that mediate the measured effect. In practice, measurement of drug concentration at the receptor is often difficult, and the site of receptors might be unknown or broadly distributed. Accordingly, a common compromise is to measure drug concentrations in more accessible compartments (e.g., venous blood or cerebrospinal fluid) that usefully approximate drug concentrations across broad areas within the organism.

A second advantage of PKPD analysis is that it permits evaluation of the relationship between drug effect and concentrations not only of the administered drug, but also of drug metabolites. All drugs are subject to at least some degree of metabolism in the body, and in many cases, these metabolites are active and may contribute to the overall effect produced by an administered drug dose. An extreme example of this phenomenon is prodrugs, which are compounds designed to be metabolized in the body to active metabolites that then produce the drug's intended effect [2]. When samples of blood or cerebrospinal fluid are collected and analyzed for concentrations of the administered drug, they can also be analyzed for concentrations of known or suspected metabolites, and changing drug effects over time can be related to changing concentrations of the metabolites as well as of the parent drug.

A third advantage of PKPD analysis is that it provides a basis not only for evaluating changing drug effects over time within an organism, but also for evaluating variable drug effects between organisms [3]. Thus, the administration of a given drug dose in mg/kg units often produces different effects across subjects within a species or across subjects of different species in translational studies. One factor that may contribute to such between-subject or between-species variability in drug effect is variability in PK processes. For example, metabolism may proceed at different rates or yield different metabolites in different subjects, and these differences in metabolism will result in different temporal profiles of drug and metabolite concentrations and associated behavioral and physiological effects despite use of the same administered dose. Use of drug and metabolite concentration, rather than drug dose, as the primary independent variable can reveal PK differences across subjects or species and provide a basis for integrating these differences into interpretation of drug effects.

The remainder of this chapter will illustrate strategies for using PKPD analysis in drug discrimination research using results from studies in rhesus monkeys trained to discriminate cocaine from saline.

2 PKPD Analysis of the Discriminative Stimulus Effects of Cocaine

2.1 PKPD Analysis in Rhesus Monkeys

Cocaine produces reliable discriminative stimulus effects in rhesus monkeys and other species, and these effects are both dose- and time-dependent. As one example, Fig. 1a shows the time course of the cocaine training dose in rhesus monkeys trained to discriminate 0.4 mg/kg intramuscular cocaine from saline in a two-key, food-reinforced drug discrimination procedure [4]. During training sessions, either cocaine or saline was administered 10 min before a 5-min response period, and only responding on the injection-appropriate lever produced food. During time-course test sessions (separate test sessions for each pretreatment time), the cocaine training dose was administered 1, 3, 5, 10, 20, 30, 60, or 100 min before 5-min response periods, during which responding on either key produced food. Under these conditions, the discriminative stimulus effects of cocaine displayed a rapid onset of action, peaking within 3 min, and had a relatively short duration of action, with effects declining after 20 min and no longer apparent after 100 min. Figure 1b shows venous plasma levels of cocaine from these same monkeys. Samples were collected separately from behavioral studies, and for plasma collection, subjects were anesthetized with ketamine, equipped with a temporary catheter in the saphenous vein, and placed into a primate restraint chair. The training dose of 0.4 mg/kg cocaine was administered intramuscularly as in behavioral sessions, and samples were collected at the same times as the onset of response periods in behavioral sessions. Venous cocaine levels peaked after 10 min and then declined. Figure 1c directly compares the time course of discriminative stimulus effects and venous cocaine levels after administration of 0.4 mg/kg cocaine, and for this figure, "Time" on the X-axis is represented on a log scale to facilitate comparison of effects that occurred early as well as later after cocaine administration. This comparison shows that both the onset and offset of cocaine-induced discriminative stimulus effects occurred earlier than the rise and fall in venous cocaine levels. Lastly, Fig. 1d shows a plot of discriminative stimulus effect as a function of venous cocaine levels over time, and arrows show the sequence in which data points were collected from first to last. This plot shows a variable relationship over time between venous cocaine levels and levels of cocaine-appropriate responding. For example, similar venous cocaine levels of 35–40 ng/ml were associated with nearly 100% cocaine-appropriate responding after 3 min but with less than 25% cocaine-appropriate responding after 60 min. This type of data display is known as a "hysteresis loop," with the term "hysteresis" denoting a changing relationship over time between drug concentration and drug effect, and the term "loop" denoting the circular shape of the graph. Moreover, the direction of the loop can also be specified, and in this case, the loop is clockwise (i.e., the trajectory of data points over time flows in a clockwise direction).

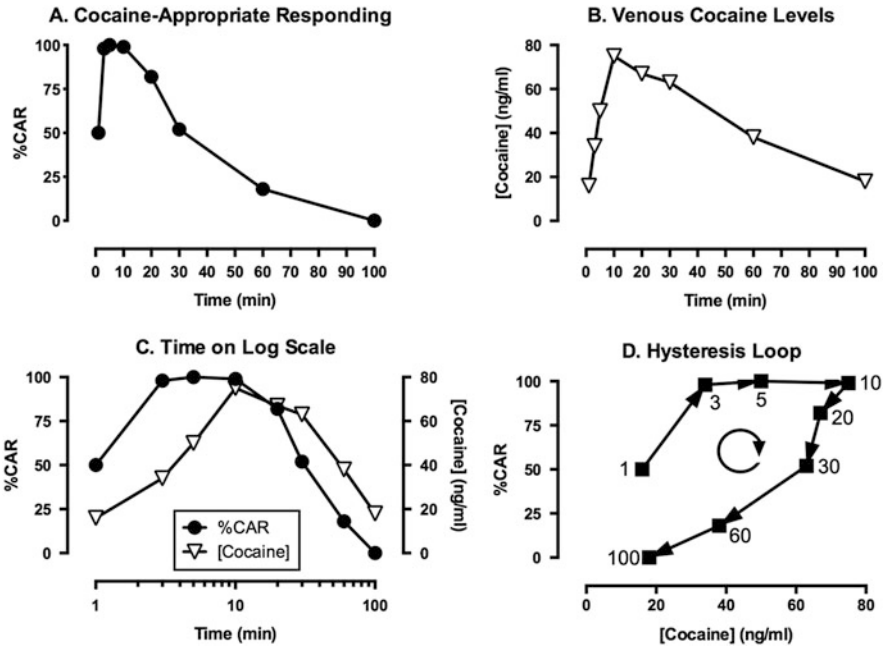

Fig. 1 PKPD analysis of discriminative stimulus effects produced by 0.4 mg/kg intramuscular cocaine in rhesus monkeys. (**a**) Time course of discriminative stimulus effects expressed as % Cocaine-Appropriate Responding (%CAR). (**b**) Time course of venous cocaine concentrations expressed in units of ng/ml of plasma. (**c**) Comparison of the time course of discriminative stimulus effects and venous cocaine levels with time expressed on a log scale. (**d**) Concentration-effect relationship between venous cocaine levels and %CAR and the resulting clockwise hysteresis loop. *Numbers* indicate the time in minutes after cocaine injection, and the clockwise circular *arrow* indicates the clockwise flow of data in the hysteresis loop. Adapted from Lamas et al. [4]

2.2 Relationship to PKPD Analysis in Humans

Hysteresis loops are common in PKPD analyses, and the presence and direction of the loop (clockwise or counterclockwise) can be used to draw inferences about PK and PD processes that contribute to drug effects in whole organisms [5]. At the most superficial level of analysis, the clockwise hysteresis loop observed in Fig. 1d for the relationship between venous cocaine levels and cocaine-induced discriminative stimulus effects in monkeys indicates that discriminative stimulus effects declined faster than venous drug concentrations. Before addressing the implications of this finding in more depth, it is first useful to note that these results in monkeys agree with the observation of clockwise hysteresis loops for cocaine-induced subjective effects in humans [6, 7]. For example, Evans et al. evaluated the time course of venous cocaine levels and a range of subjective effects after either smoked cocaine (25 or 50 mg) or intravenous cocaine (16 or 32 mg) in human subjects experienced

with both routes of cocaine administration [7]. Of relevance to this review, both smoked and intravenous cocaine yielded clockwise hysteresis loops relating venous cocaine levels to subjective effects such as "Stimulated," "High," and "Drug Liking." In addition to this qualitative similarity, results of these studies in monkeys and humans could also be compared quantitatively. Specifically, intramuscular administration of 0.4 mg/kg cocaine in monkeys (equivalent to 28 mg in a 70 kg human) produced venous cocaine concentrations that were similar in magnitude, though with a slightly delayed time course, to those produced by 25–50 mg of smoked cocaine in humans, and both produced about half of the peak venous cocaine concentrations produced in humans by intravenous 16–32 mg cocaine. The delayed time course is consistent with the slower rate of drug absorption by the intramuscular route of cocaine administration used in monkeys than by the inhalation and intravenous routes used in humans. However, the finding that similar venous cocaine levels produced discriminative stimulus effects in monkeys and subjective effects in humans provides additional evidence for similarities between discriminative effects of drugs in animals and subjective drug effects in humans.

2.3 PK Factors in the Clockwise Hysteresis Loop for Cocaine

In addition to providing a nuanced basis for evaluating translation of drug effects across species, concentration-effect curves and hysteresis loops can also provide additional insights into the pharmacological determinants of drug effects in general and discriminative stimulus effects in particular. Table 1 lists some of the processes that may contribute to clockwise or counterclockwise hysteresis loops. In the case

Table 1 Factors that may contribute to clockwise and counterclockwise hysteresis loop

I. Clockwise hysteresis
A. Pharmacokinetic factors
1. Slower distribution to site of drug concentration measurement than to site of drug action
2. Generation of an active antagonistic metabolite
B. Pharmacodynamic factors (acute tolerance/tachyphylaxis)[a]
1. Rates of receptor binding or signal transduction much faster than rates of drug distribution
2. Desensitization/downregulation of receptors or downstream signaling pathways over time
3. Recruitment of negative feedback processes
II. Counterclockwise hysteresis
A. Pharmacokinetic factors
1. Faster distribution to site of drug concentration measurement than to site of drug action
2. Generation of an active agonist metabolite (e.g., by a prodrug)
B. Pharmacodynamic factors[a]
1. Rates of receptor binding or signal transduction much slower than rates of drug distribution
2. Sensitization/upregulation of receptors or downstream signaling pathways over time
3. Recruitment of positive feedback processes

[a]Listed PD factors apply for drugs that are agonists at their target receptor. For drugs that function as antagonists or inhibitors, different mechanisms would apply. See text for a discussion of mechanisms that might apply for cocaine

of cocaine discrimination shown in Fig. 1, at least two factors appear to contribute to the clockwise hysteresis loop that relates venous cocaine levels to discriminative stimulus effects.

First, regarding PK, recall that a drug is absorbed from its site of administration and distributed through a circuit of compartments before it is ultimately metabolized and/or excreted. For example, Fig. 2 shows that intramuscular cocaine is absorbed into the blood stream in muscle and transferred by veins to the heart and cardiopulmonary circulatory system where blood is oxygenated. After oxygenated blood containing cocaine returns to the heart, it is pumped via the aorta and systemic arteries to sites throughout the body, including sites of drug action such as brain, before being collected in veins and returned to the heart and cardiopulmonary system. Metabolism and excretion can occur at multiple points along this circuit. For cocaine, metabolism occurs largely via esterases in blood and liver, and excretion of cocaine and metabolites occurs largely via the kidneys into urine [9]. For the study shown in Fig. 1, cocaine-induced discriminative stimulus effects are thought to be mediated largely by binding of cocaine to dopamine transporters at one location (i.e., brain; [10, 11]), and cocaine concentrations were determined in plasma isolated from a different location (i.e., venous blood samples collected from the saphenous vein). As depicted in Fig. 2, intramuscular cocaine would be distributed to its site of action in brain before it would reach the saphenous vein and other systemic veins, and this lag in drug distribution from the site of cocaine action to the site of blood collection could contribute to the lag between expression of discriminative stimulus effects and the later increases in venous cocaine levels. Results from the study by Evans et al. in humans support this possibility [7]. In addition to measuring subjective effects and venous cocaine levels after cocaine administration in humans, these authors also measured arterial cocaine levels, and thereby sampled cocaine concentrations from compartments that would be reached before as well as after access of cocaine to its sites of action in brain. Arterial cocaine levels peaked at approximately tenfold higher concentrations than venous levels, these peaks were reached more quickly in arteries than in veins (15 s vs. 4 min for both smoked and intravenous cocaine), and the arterial concentration-effect curve resulted in a counterclockwise rather than a clockwise hysteresis loop. A similar finding for

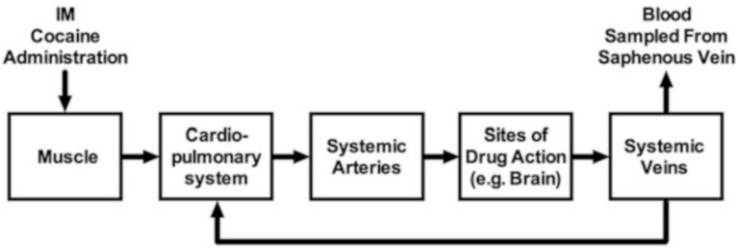

Fig. 2 Schematic of drug distribution after intramuscular drug injection. For the studies shown in Figs. 1 and 3, drug and metabolite concentrations were determined from plasma of blood samples collected from the saphenous vein

counterclockwise vs. clockwise hysteresis loops has also been found for arterial vs. venous concentration-effect curves for the short-acting opioid remifentanil, which is also rapidly metabolized by esterases in blood [12]. These results are consistent with drug distribution first to arteries, then to sites of action in brain, and lastly to veins. Additionally, rapid metabolism to inactive metabolites contributes to markedly lower concentrations reaching venous blood, thereby accentuating the expression of clockwise hysteresis loops that relate centrally mediated drug effects to venous drug levels.

2.4 PD Factors in the Clockwise Hysteresis Loop for Cocaine

Although PK factors likely bear primary responsibility for the clockwise hysteresis loop relating venous cocaine levels to discriminative stimulus subjective effects for cocaine, PD factors may also contribute. In particular, clockwise hysteresis loops are suggestive of acute tolerance to drug effects. "Tolerance" is a descriptive rather than an explanatory term, and in the context of PKPD analysis, it indicates a decrease in effect produced by a given drug concentration over time without implicating a particular mechanism. Possible mechanisms that may contribute to the phenomenon of acute tolerance (also known as "tachyphylaxis") are listed in Table 1. Insofar as cocaine produces its discriminative stimulus effects primarily by blocking dopamine transporters and increasing extracellular dopamine levels in brain regions such as nucleus accumbens [10, 11, 13], possible mechanisms of acute tolerance to the discriminative stimulus effects of cocaine could result from upregulation of dopamine transporters to facilitate dopamine clearance, decreases in dopamine release due to feedback inhibition of dopamine neuronal activity, and/or desensitization or downregulation of postsynaptic dopamine receptors responding to elevated dopamine levels. The precise mechanisms that might confer acute tolerance during the time course of effects pursuant to a single cocaine administration remain to be fully elucidated. However, acute tolerance has been observed for many cocaine effects in experimental designs that involve two sequential cocaine treatments. For example, pretreatment with an active cocaine dose in humans decreased the cardiovascular and subjective effects of a second cocaine dose administered 60 min later [14], and in rhesus monkeys, two similarly spaced cocaine injections resulted in a smaller increase in extracellular dopamine levels in nucleus accumbens after the second injection [15].

3 PKPD Analysis of the Cocaine-Like Discriminative Stimulus Effects of Lisdexamfetamine and Phendimetrazine

3.1 Lisdexamfetamine

Lisdexamfetamine is a prodrug for D-amphetamine in which the amino acid L-lysine is coupled to the nitrogen of amphetamine [16, 17]. It is approved for treatment of attention-deficit hyperactivity disorder and binge-eating disorder, and it is also under consideration as a maintenance medication for treatment of cocaine abuse [8, 18]. Lisdexamfetamine is thought to be inactive as a parent drug, but it is metabolized in blood to lysine and the active metabolite amphetamine by peptidase enzymes associated with red blood cells [19]. Administration of amphetamine itself substitutes for the discriminative stimulus effects of cocaine across a wide range of conditions (e.g., [20]), and Fig. 3 shows results from a study that examined effects of lisdexamfetamine in rhesus monkeys trained to discriminate 0.32 mg/kg intramuscular cocaine from saline in a procedure otherwise identical to the one described above for studies with cocaine [8]. Lisdexamfetamine produced a dose- and time-dependent substitution for cocaine, and Fig. 3a shows the time course of cocaine-like discriminative stimulus effects produced by a dose of 3.2 mg/kg lisdexamfetamine, together with venous plasma levels of lisdexamfetamine and D-amphetamine. The discriminative stimulus effects of lisdexamfetamine had a slow onset and long duration of action. Venous levels of lisdexamfetamine were highest at the initial measurement at 10 min and declined rapidly to low levels, whereas venous amphetamine levels peaked more slowly and declined more gradually over a period of 2 days. The delayed appearance of amphetamine is consistent with the conclusion that amphetamine is a metabolite of lisdexamfetamine.

Figure 3b shows the hysteresis loop relating discriminative stimulus effects to venous levels of the parent compound lisdexamfetamine, and this hysteresis loop differs from that for cocaine in Fig. 1d in two ways. First, the initial rise in %CAR was not associated with a parallel rise in venous lisdexamfetamine levels. Rather, the highest lisdexamfetamine levels measured at 10 min were associated with low levels of cocaine-appropriate responding, and the onset of discriminative stimulus effects was associated with a drop in venous lisdexamfetamine levels. Second, the hysteresis loop flowed in a counterclockwise rather than in a clockwise direction. These two phenomena together are consistent with the conclusion that lisdexamfetamine is an inactive prodrug being converted to an active metabolite [5].

Figure 3c shows the hysteresis loop relating discriminative stimulus effects to venous plasma levels of amphetamine. In contrast to the plot for the parent drug, the plot for amphetamine did show rising plasma levels during the onset of discriminative stimulus effects during the first 30 min after drug administration, suggesting that amphetamine is indeed functioning as an active metabolite of lisdexamfetamine. However, as with the parent drug, the overall hysteresis loop

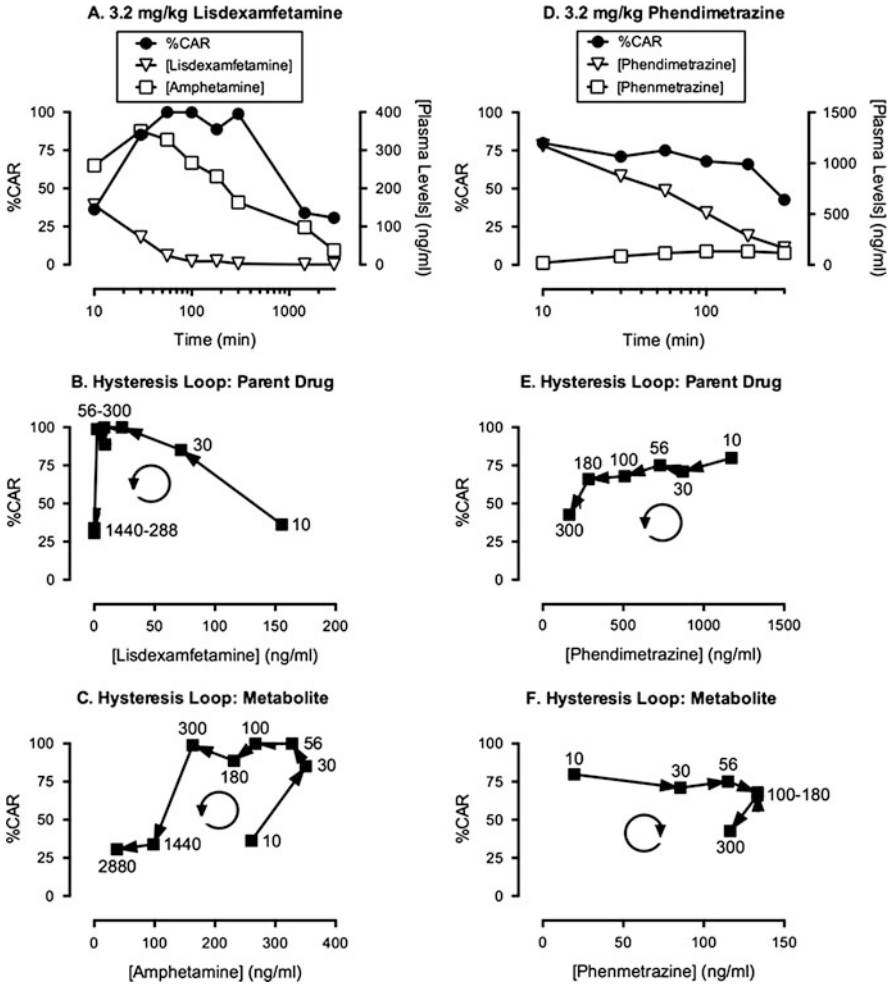

Fig. 3 PKPD analysis of cocaine-like discriminative stimulus effects produced by intramuscular lisdexamfetamine and phendimetrazine in rhesus monkeys. (**a**, **d**) Time course of discriminative stimulus effects (expressed as % Cocaine-Appropriate Responding; %CAR) and venous plasma levels of the parent drug and metabolite (ng/ml) for lisdexamfetamine (**a**) or phendimetrazine (**d**). Note that time in minutes is shown on a log scale. (**b**, **e**) Hysteresis loops for venous levels of the parent drug and %CAR for lisdexamfetamine (**b**) and phendimetrazine (**e**). (**c**, **f**) Hysteresis loops for venous levels of the metabolite and % CAR for lisdexamfetamine (**c**, metabolite = amphetamine) and phendimetrazine (**f**, metabolite = phenmetrazine). Numbers in (**b**, **c**, **e**, and **f**) indicate the time in minutes after parent drug injection, and the circular *arrows* indicate the clockwise or counterclockwise flow of data in the hysteresis loop. Adapted from Banks et al. [8]

for amphetamine also flowed in a counterclockwise direction. A counterclockwise hysteresis loop was also reported for the relationship for venous amphetamine levels to locomotor activity and mesolimbic dopamine release in rats after lisdexamfetamine administration [21]. This observation has been interpreted to

suggest that amphetamine levels accumulate in systemic vasculature in general, and systemic veins in particular, more quickly than in brain to produce centrally mediated effects [8, 21]. More specifically, in reference to Fig. 2, these findings suggest that most of the conversion of lisdexamfetamine to amphetamine occurs in systemic veins. This conclusion would be consistent with (1) the requirement for peptidases in red blood cells to accomplish this metabolism, (2) the higher percentage of total blood volume in veins vs. arteries, and (3) the consequent longer residence time for any one circulating blood constituent (e.g., a red blood cell or drug molecule) in veins vs. arteries. Any amphetamine generated from lisdexamfetamine in veins would then require recirculation for delivery to brain. Moreover, rates of amphetamine delivery from vasculature across the blood–brain barrier and into neural tissue may also be limited [21], and this would produce a further delay between the time course of venous amphetamine levels and the time course of discriminative stimulus effects.

Two other points warrant mention. First, the venous amphetamine levels associated with cocaine-like discriminative stimulus effects in monkeys are much higher after lisdexamfetamine administration than after administration of amphetamine itself. For example, Fig. 3a shows that the dose of 3.2 mg/kg lisdexamfetamine sufficient to produce full substitution yielded a peak venous amphetamine levels of more than 300 ng/ml, whereas a dose of 0.32 mg/kg amphetamine sufficient to produce full substitution produced peak venous amphetamine levels of less than 100 ng/ml (M.L. Banks and S.S. Negus; unpublished results), and an oral dose of 20 mg amphetamine sufficient to produce significant subjective effects in humans yielded peak venous plasma levels of approximately 40 ng/ml [22]. One likely explanation for this difference is that venous levels after amphetamine administration likely underestimate the arterial drug levels initially delivered to the site of action (e.g., see above for cocaine), whereas venous levels after lisdexamfetamine are likely very similar to arterial levels delivered to the site of action (because amphetamine is generated largely in the systemic venous compartment). Direct evaluation of this hypothesis would be useful by comparing venous and arterial levels of amphetamine after lisdexamfetamine administration. In a second and related point, counterclockwise hysteresis loops relating venous amphetamine levels and centrally mediated behavioral effects after lisdexamfetamine administration differ from the finding of clockwise hysteresis loops after administration of amphetamine itself. For example, oral amphetamine in humans results in clockwise hysteresis loops that relate venous amphetamine levels to subjective effects [22], and we have similarly found that intramuscular amphetamine in rhesus monkeys produces clockwise hysteresis loops that relate venous amphetamine levels to cocaine-like discriminative stimulus effects (M.L. Banks and S.S. Negus, unpublished results). This distinction in rotational direction for hysteresis loops for amphetamine administered either directly or generated via metabolism of lisdexamfetamine illustrates one manifestation of PK differences that can be produced by different formulations of the same drug. In this case, the implication is that administration of amphetamine itself results in distribution of drug to sites of drug action before delivery to systemic veins, whereas

administration of lisdexamfetamine results in generation of amphetamine in systemic veins prior to its delivery to sites of drug action.

3.2 *Phendimetrazine*

Phendimetrazine is approved for clinical use as an appetite suppressant for the treatment of obesity [23], and like lisdexamfetamine, it is also under consideration as a maintenance medication for the treatment of cocaine use disorder [24]. Phendimetrazine is metabolized to the compound phenmetrazine, and although both drugs interact with dopamine and norepinephrine transporters, the metabolite has high potency and functions as an amphetamine-like transporter substrate that promotes release of dopamine and norepinephrine, whereas the parent compound is more than 100-fold less potent and functions as a cocaine-like transporter inhibitor that prevents dopamine and norepinephrine reuptake [25]. The low potency of phendimetrazine at monoamine transporters suggested that it might function as a relatively inactive prodrug for the active metabolite phenmetrazine, similar to the function of lisdexamfetamine as a prodrug for amphetamine. This hypothesis was tested in PKPD studies in cocaine-discriminating rhesus monkeys [26]. For the purposes of the discussion below, phendimetrazine will be referred to as PDM, and phenmetrazine will be referred to as PM, because the spellings of the full drug names are similar and easily confused.

Initial studies indicated that administration of PM directly produced dose- and time-dependent substitution for cocaine and increases in venous PM levels, and the hysteresis plot relating venous PM concentration to cocaine-appropriate responding rotated in a clockwise direction similar to that described above for cocaine and amphetamine. PDM also produced dose- and time-dependent substitution for cocaine, and Fig. 3d shows results with a dose of 3.2 mg/kg PDM. Figure 3d also shows that this PDM dose produced time-dependent increases in venous levels of both PDM and PM. PDM levels peaked at the earliest time point at levels greater than 1,000 ng/ml, whereas PM levels rose more slowly and peaked at tenfold lower levels of approximately 100 ng/ml. The delayed emergence of PM after PDM administration is consistent with the status of PM as a metabolite of PDM. Moreover, venous PM levels were similar after administration of behaviorally active doses either of PM itself or of PDM, consistent with the conclusion that PM was functioning as an active metabolite sufficient to mediate behavioral effects of PDM. However, the PKPD profile of PDM and its metabolite PM differed from the profile for lisdexamfetamine and its metabolite amphetamine in two ways as illustrated by the hysteresis plots.

First, Fig. 3e shows the hysteresis loop that relates venous PDM levels to cocaine-appropriate responding. As with lisdexamfetamine, the direction of rotation for this hysteresis loop was counterclockwise; however, in contrast to results with lisdexamfetamine, the highest venous levels of PDM were associated with the highest levels of cocaine-appropriate responding. Although earlier time points were

not assessed, these results indicate that the onset of cocaine-appropriate responding was associated with the period of rising PDM levels.

Second, Fig. 3f shows the hysteresis loop that relates PM levels to cocaine-appropriate responding. In contrast to the findings for amphetamine after lisdexamfetamine administration, the hysteresis loop for PM after PDM administration rotated in a clockwise direction. Of particular importance, high levels of cocaine-appropriate responding were observed at the earliest time point when PM levels were low, and the period of rising PM levels was associated not with onset of cocaine-appropriate responding, but rather with a period of sustained cocaine-appropriate responding. At later time points, there was a decrease in both venous PM levels and in cocaine-appropriate responding.

Taken together, these results were not consistent with the conclusion that PDM was an inactive parent drug for the active metabolite PM. Rather, these findings suggest that both PDM and PM were active, and the time course of cocaine-like discriminative stimulus effects after PDM administration reflected an initial phase of cocaine-like effects mediated by the parent drug PDM followed by a later phase of cocaine-like effects mediated by the metabolite PM.

4 Conclusions

PKPD analysis is an alternative to conventional dose-effect analysis of in vivo drug effects, and it focuses on the relationship of drug-induced behavioral or physiological effects to drug and metabolite concentrations in the body rather than to drug dose. Hysteresis loops are one manifestation of PKPD analysis, and these loops describe the time course of the potentially variable relationship between drug/metabolite concentration and drug effect over time. PKPD analysis, including analysis of hysteresis loops, can play a valuable role in interpretation of drug effects and PKPD relationships for the purposes of drug assessment and translational research in pharmacology. This chapter provided examples of the application of PKPD analysis to studies of the discriminative stimulus effects of drugs.

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Human Drug Discrimination: Elucidating the Neuropharmacology of Commonly Abused Illicit Drugs

B. Levi Bolin, Joseph L. Alcorn, Anna R. Reynolds, Joshua A. Lile, William W. Stoops, and Craig R. Rush

Abstract Drug-discrimination procedures empirically evaluate the control that internal drug states have over behavior. They provide a highly selective method to investigate the neuropharmacological underpinnings of the interoceptive effects of drugs in vivo. As a result, drug discrimination has been one of the most widely used assays in the field of behavioral pharmacology. Drug-discrimination procedures have been adapted for use with humans and are conceptually similar to preclinical drug-discrimination techniques in that a behavior is differentially reinforced contingent on the presence or absence of a specific interoceptive drug stimulus. This chapter provides a basic overview of human drug-discrimination procedures and reviews the extant literature concerning the use of these procedures to elucidate the underlying neuropharmacological mechanisms of commonly abused illicit drugs (i.e., stimulants, opioids, and cannabis) in humans. This chapter is not intended to review every available study that used drug-discrimination procedures in humans. Instead, when possible, exemplary studies that used a stimulant, opioid, or Δ^9-tetrahydrocannabinol (the primary psychoactive constituent of cannabis) to assess the discriminative-stimulus effects of drugs in humans are reviewed

B.L. Bolin, J.L. Alcorn, and A.R. Reynolds
Department of Behavioral Science, University of Kentucky College of Medicine, 140 Medical Behavioral Science Building, Lexington, KY 40536-0086, USA

J.A. Lile, W.W. Stoops, and C.R. Rush (✉)
Department of Behavioral Science, University of Kentucky College of Medicine, 140 Medical Behavioral Science Building, Lexington, KY 40536-0086, USA

Department of Psychology, University of Kentucky College of Arts and Sciences, 110 Kastle Hall, Lexington, KY 40506-0044, USA

Department of Psychiatry, University of Kentucky College of Medicine, 3470 Blazer Parkway, Lexington, KY 40509, USA
e-mail: crush2@email.uky.edu

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for illustrative purposes. We conclude by commenting on the current state and future of human drug-discrimination research.

Keywords Abuse potential • Amphetamines • Cannabis • Cocaine • Drug discrimination • Humans • Medications development • Neuropharmacology • Opioids • Pharmacotherapy • Subject-rated effects • Substance abuse • THC

Contents

1 Introduction

Drug-discrimination procedures empirically evaluate the control internal drug states have over behavior. They provide a highly selective method to investigate the neuropharmacological underpinnings of the interoceptive effects of drugs in vivo. As a result, drug discrimination has been one of the most widely used assays in the field of behavioral pharmacology. Since the publication of one of the earliest studies to suggest the control of behavior by the presence or absence of the interoceptive-stimulus effects of alcohol in rats [1], there has been substantial work investigating the discriminative-stimulus effects of drugs spanning more than four decades (e.g., [2]). Drug-discrimination procedures have also been adapted for use with humans and remain conceptually similar to preclinical drug-discrimination procedures in that a behavior is differentially reinforced contingent on the presence or absence of a specific interoceptive drug stimulus (see Chap. 1; also see [3]). A PubMed search using the quoted search phrase “drug discrimination” yields 1,284 peer-reviewed publications dating back to the mid-1940s (i.e., [4]). Of the total number of published drug-discrimination studies, those concerning human drug discrimination comprise approximately 16% (i.e., 205 reports). Figure 1 shows the total number of drug-discrimination publications per year since 1973 and the relative proportion of those concerning human drug discrimination.

As noted above and described in previous chapters, the interoceptive-stimulus effects of drugs and the ensuing stimulus control of behavior have been widely studied in non-human laboratory animals using drug-discrimination procedures.

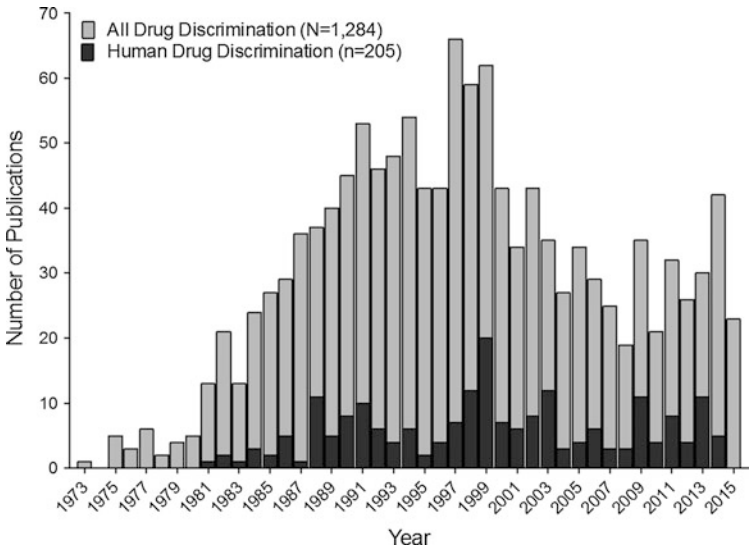

Fig. 1 Number of published drug-discrimination reports per year from 1973 to 2015. The total number of drug-discrimination publications is shown in *light gray* bars. The relative number of published drug-discrimination studies involving human participants is shown in *dark gray* bars. *X*-axis: Publication Year. *Y*-axis: Number of Publications

Below, the extant literature that assessed the discriminative-stimulus effects of stimulants, opioids, and Δ^9-tetrahydrocannabinol (Δ^9-THC; the primary pharmacological constituent in cannabis) 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 (e.g., [5–7]); (b) the concordance between preclinical and human drug-discrimination experiments [8]; and (c) the neuropharmacological selectivity of drug-discrimination procedures relative to subjective drug-effect questionnaires [9]. Although the present chapter provides some general discussion of these previously reviewed topics, it differs from earlier reviews in that it primarily focuses on the utility of human drug-discrimination procedures to elucidate the underlying neuropharmacological mechanisms of commonly abused illicit drugs (i.e., stimulants, opioids, and cannabis). This chapter is not intended to review every available study that used human drug-discrimination procedures. Instead, when possible, studies that used a stimulant, opioid, or Δ^9-THC to assess the discriminative-stimulus effects of drugs in humans are reviewed for illustrative purposes. Lastly, we conclude by commenting on the current state and future of human drug-discrimination research.

1.1 Subject Recruitment and Selection

Potential subjects are typically recruited through formal advertisements in local newspapers, online classified ads (e.g., Craigslist), flyers posted in public areas, and by word-of-mouth referral. Volunteers who may qualify upon initial screening complete a rigorous in-person screening that includes a complete medical history, physical health screen, and psychiatric assessment. Volunteers also provide basic demographic information (e.g., age, sex, and socioeconomic status) and complete a battery of questionnaires that assess drug-use history and severity as well as symptomology for other clinically relevant conditions such as depression and attention-deficit hyperactivity disorder. Responses on these instruments are used to determine whether volunteers satisfy the study inclusion criteria or meet criteria that would exclude them from participation (e.g., active disease process, psychiatric disorder, and prescribed medication(s) contraindicated with the study medication). Given the substantial time commitment required by human drug-discrimination studies, another important consideration is whether a potential subject is able to dedicate the time necessary to complete the study. A physician reviews all screening materials to determine whether the volunteer is physically and psychologically eligible for participation. Thorough physical and mental health screening is absolutely imperative to ensure subject safety in any study involving the administration of pharmacological agents to human subjects.

The discriminative-stimulus effects of various drugs have been assessed in normal healthy volunteers (e.g., [10, 11]), drug-dependent individuals (e.g., [12, 13]), and individuals with a history of drug dependence who are currently abstinent/detoxified (e.g., [14]). However, there are no published studies in which the discriminative-stimulus effects of particular drugs have been prospectively compared between these populations. Several factors should be considered when selecting the most appropriate population of subjects given the specific research question(s) and the primary aim(s) of the study. For example, participants with an extensive history of substance abuse may be most appropriate in the context of testing whether a novel compound has potential for abuse itself or may effectively attenuate the discriminative-stimulus effects of a drug with known abuse potential. An important caveat, however, is that their extensive drug-use history may complicate interpretation of the results because of differences in expectancies, conditioning history, and tolerance [15]. Although there are advantages and disadvantages to using various populations, research in individuals with and without histories of substance abuse is necessary to gain a more complete understanding of the neuropharmacological mechanisms that underlie the discriminative-stimulus effects of drugs [15].

Test Environment and Experimental Materials and Methods

The test environment and experimental materials required to conduct a human drug-discrimination experiment generally consists of a test room containing a desk, chair, a computer with a mouse, numeric keypad and programming to present the drug-discrimination task and record the data, and equipment that is used to monitor participants' vital signs. Although the use of a computer is more typical, pen and paper could also be used for task presentation and data collection. The room may also be equipped with a television and other recreational materials (e.g., magazines, books, games, and craft supplies) that volunteers may use when not engaged in experimental activities.

In one example of a two-choice drug-discrimination task, the volunteer is presented with two response options (e.g., Drug A and Not Drug A) on the computer screen and instructed to indicate which drug condition that they think they received by distributing 100 points between the two options using the numeric keypad. For example, if a volunteer is relatively confident that they received Drug A, they might allocate 80 points to the Drug A option and 20 points to the Not Drug A option. Volunteers complete the drug-discrimination task multiple times at regular intervals throughout the session: usually every 30 min to an hour depending on the pharmacokinetics of the drug(s) under study. The total number of points allocated to the correct response option out of all possible points is exchanged for money at a constant rate. For example, points have been exchanged for money at rate of $0.04–$0.08 per point in previous drug-discrimination studies conducted in our laboratory [16, 17]. Participants can earn $20–$40 per session but the specific rate with which points are exchanged for money (i.e., $0.04 vs. $0.08) does not appear to significantly alter performance on the task [16, 17].

The use of money as the reinforcer in human drug-discrimination studies is a primary difference from preclinical drug-discrimination studies. In preclinical studies, subjects are often food restricted so that food reinforcers effectively maintain behavior. Another notable difference between preclinical and human drug-discrimination studies is that some human studies do not utilize a formal schedule of reinforcement, at least as typically conceptualized, and reinforcement is withheld until the end of the session when subjects are paid. In contrast, responding by animals is typically maintained by a fixed-ratio schedule of reinforcement and reinforcers are delivered or withheld upon completion of each response requirement.

1.2 Human Drug Discrimination: Procedural Overview

This section of the chapter provides a general experimental overview and highlights the basic methodological elements of human drug-discrimination procedures. Notable procedural variations between drug-discrimination studies, more complex drug-

discrimination procedures, and the advantages and limitations of these approaches are then discussed. As noted above, the methods used in human drug-discrimination studies are very similar to those used in preclinical drug-discrimination research. Although a standardized human drug-discrimination procedure has not been established, these experiments often consist of three phases that are completed in a fixed order: (1) Sampling Phase; (2) Acquisition Phase; and (3) Test Phase.

Sampling Phase During the sampling phase, participants complete several experimental sessions to acquaint them with the interoceptive-stimulus effects of the training dose. The training dose is usually identified to participants by a specific 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 attend to the effects of the drug because correctly identifying the drug they received will determine the amount of monetary compensation that they earn in future sessions.

Acquisition Phase Following the sampling phase, an acquisition phase (sometimes referred to as the test-of-acquisition or control phase) is conducted in which the training dose and placebo are administered once per day across several sessions (e.g., 4–12 total sessions) in random order. During each session in this phase, volunteers ingest drug or placebo under blinded conditions and then complete the drug-discrimination task along with subjective drug-effect questionnaires periodically for several hours after drug administration. Although participants are asked to identify which treatment they received on the drug-discrimination task periodically throughout the session, the correct treatment code (i.e., Drug A vs. Not Drug A; Drug A vs. Drug B; and Red Drug vs. Blue Drug) is not revealed to the participant until the conclusion of the session. The percentage of correct responses (i.e., correct identification of the treatment) is then converted to money and the participant is told immediately how much bonus money they earned during the experimental session. The performance 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 beyond the acquisition phase. The extensive training associated with human drug-discrimination procedures provides participants with similar recent behavioral and pharmacological histories, which is thought to reduce variability both within and across participants.

Test Phase The final phase is the test phase, during which the discriminative-stimulus effects of different doses of the training drug, novel drugs, or drug combinations are determined. Sessions involving the administration of doses or drugs other than the training condition are deemed to be "test sessions." Participants are not told the purpose of test sessions, nor do they know when these sessions are scheduled until completing the session. As is the case in preclinical studies, there is no correct response per se during these test sessions, so participants usually receive all of the available money that is contingent on correctly identifying the drug

condition that was administered. Test-of-acquisition sessions that are identical to those in the acquisition phase are interspersed among test sessions to ensure that participants continue to accurately discriminate the training dose versus placebo. Additional sessions are inserted to re-establish accurate discrimination if the participant fails to correctly identify the training condition they received during a test-of-acquisition session conducted during the test phase. The number of test-of-acquisition sessions included in the test phase varies but is usually fewer than the total number of test sessions (e.g., 25–50%).

In general, there are two strategies in the choice of drug conditions administered in the test phase with the goal of elucidating the neuropharmacological mechanisms that mediate the discriminative-stimulus effects of the training drug. The first is the use of substitution procedures, in which a range of doses of other drugs is tested to determine if they share discriminative-stimulus effects with the training drug. Based on the drugs that produce significant drug-appropriate responding, inferences can be made regarding the neuropharmacological mechanisms that mediate the effects of the training drug. The second approach is to determine a dose–response curve for the training drug alone and in combination with pharmacologically selective compounds. These compounds can be administered concurrently with the training drug or one given as a pretreatment to the other, depending on the pharmacokinetic profiles of the training and test drugs. Inferences are made regarding the neuropharmacological mechanisms that mediate the discriminative-stimulus effects of the training drug based on the mechanism of action of the test drugs that shift the training-drug dose–response curve.

Advantages and Limitations of Human Drug-Discrimination Procedures Human drug-discrimination procedures offer a number of advantages relative to other assays commonly used in behavioral pharmacology. As mentioned previously, three strengths of human drug discrimination are that it produces data that are orderly and dose-dependent, is pharmacologically selective, and that subjects have virtually identical training and recent drug-exposure histories prior to testing novel drugs and/or drug doses. In addition to these strengths, the relationship between the subjective- and discriminative-effects of drugs may be directly evaluated in human drug-discrimination studies.

Despite these notable strengths, human drug-discrimination procedures also have several potential limitations that warrant consideration. First, drug-discrimination procedures require extensive training before testing can begin and require a considerable investment of time and resources on the part of both volunteers and investigators. An offsetting strength is that fewer subjects are required to achieve adequate statistical power in drug-discrimination studies relative to other procedures that rely more heavily on subjective-effects measures. Second, drug-discrimination tasks specifically provide a relatively limited amount of information (i.e., typically a single outcome measure such as discrimination accuracy) as compared to other behavioral measures that provide information across an array of dimensions (e.g., subjective-effects measures; [9]). However,

the interpretation of drug-discrimination data is somewhat less complicated because conclusions may be drawn directly from performance on the discrimination task. The likelihood of Type I errors is also decreased because drug-discrimination procedures rely on a single primary-outcome measure. Third, drug-discrimination performance is relatively insensitive to changes in circulating levels of drug across the time-course of drug effects in that the allocation of responses to the drug-appropriate option does not typically decrease as blood levels decrease (e.g., [18]). Fourth, the investigation of the specific role of various molecular sites of action (e.g., transporters, receptor systems, and specific receptor subtypes) to the discriminative-stimulus effects of drugs in humans are relatively limited because medications that are approved for use with humans by the US Food and Drug Administration are typically used in human drug-discrimination studies. Fifth, as noted above, a significant challenge relative to animal models is that humans vary in their behavioral and pharmacological histories, which can affect study results and complicate the interpretation of the findings. Finally, in the context of the study of substance-use disorders, drug-discrimination procedures lack the face validity of other experimental approaches such as drug self-administration (e.g., [19]). Although the drug-discrimination paradigm may lack a certain degree of face validity relative to other experimental approaches, it has predictive validity with respect to the underlying neurobiological and neuropharmacological mechanisms of drugs and determination of the abuse potential of novel compounds (e.g., [9, 15, 20–22]).

2 Underlying Neuropharmacology of Commonly Abused Illicit Drugs

As indicated in previous chapters, drug-discrimination procedures are pharmacologically selective and, as a result, have been used to assess the underlying neuropharmacology of centrally acting drugs. In addition, findings from human drug-discrimination studies are, in many cases, consistent with the hypothesized neuropharmacological mechanisms of actions of those drugs. According to the most recent epidemiological findings, the three most-used substances in 2013 among persons age 12 years or older were cannabis (19.8 million), psychotherapeutics (including prescription stimulants and opioid pain relievers; 6.5 million), and cocaine (1.5 million; [23]). Therefore, in this section of the chapter, we have chosen to review a portion of the human drug-discrimination literature that demonstrates the utility of this behavioral assay to elucidate the underlying neuropharmacology of stimulants, opioids, and cannabis.

2.1 Stimulants

Basic Neuropharmacology and Mechanism of Action Abused stimulants exert their pharmacodynamic effects via interactions with monoamine transporters (e.g., dopamine [DA], serotonin [5-HT], and norepinephrine [NE]; reviewed in [24–27]). Prior ex vivo studies suggest that stimulants can be classified into two groups based on their differential regulation of these transporters. Amphetamines (e.g., D-amphetamine and methamphetamine) act as substrates for monoamine transporters and are transported into the nerve terminal where they prevent accumulation of neurotransmitter in storage vesicles, inhibit metabolic degradation by monoamine oxidase, and promote neurotransmitter release via carrier-mediated exchange [27]. Although amphetamines can also function as reuptake inhibitors, these effects are more moderate compared to their actions as transporter substrates [28]. By contrast, cocaine is a reuptake inhibitor and may cause firing-dependent reversal of the transporter thereby promoting the accumulation of neurotransmitter in the synapse (for a review, see [24, 29]). Central monoamine systems (e.g., DA, 5-HT and NE) are implicated in the discriminative-stimulus effects of abused stimulants [30–38]. The evidence for the involvement of central monoamine systems, namely DA, in the interoceptive effects of abused stimulants is reviewed below.

Substitution Profile Substitution tests in prior human drug-discrimination studies suggest a prominent role for central monoamine systems in the interoceptive effects of stimulants. For example, in participants discriminating D-amphetamine (i.e., 10 mg) from placebo [39], the D_2 receptor partial agonist phenylpropanolamine (i.e., 25 and 75 mg) and monoamine reuptake inhibitor mazindol (i.e., 0.5 and 2.0 mg) substituted for D-amphetamine, suggesting that central monoamine systems are critically involved in the discriminative-stimulus effects of D-amphetamine. Other studies have shown that drugs that directly modulate monoaminergic tone (e.g., caffeine and methylphenidate; [40, 41]) engender D-amphetamine-appropriate responding; whereas, drugs that do not (e.g., diazepam, hydromorphone, and diazepam) produce partial to minimal drug-appropriate responding [39, 42–50]. These studies demonstrate that D-amphetamine functions as a discriminative stimulus via complex interactions at central monoamine systems.

Central monoamine systems also play a prominent role in the discriminative-stimulus effects of methamphetamine and cocaine. In one study, participants learned to discriminate oral methamphetamine (i.e., 10 mg) from placebo [17]. A range of oral doses of methamphetamine (i.e., 2.5–15 mg), D-amphetamine (i.e., 2.5–15 mg), methylphenidate (i.e., 5–30 mg), and γ-aminobutyric acid-A ($GABA_A$) modulator triazolam (i.e., 0.0625–0.375 mg) was then tested. Figure 2 shows that D-amphetamine and methylphenidate dose-dependently increased methamphetamine-appropriate responding; whereas, triazolam failed to engender methamphetamine-appropriate responding. Similarly, Fig. 3 shows that cocaine and methylphenidate produced similar discriminative-stimulus effects in participants who had learned to discriminate oral cocaine (i.e., 150 mg) from placebo [16]. In contrast, neither

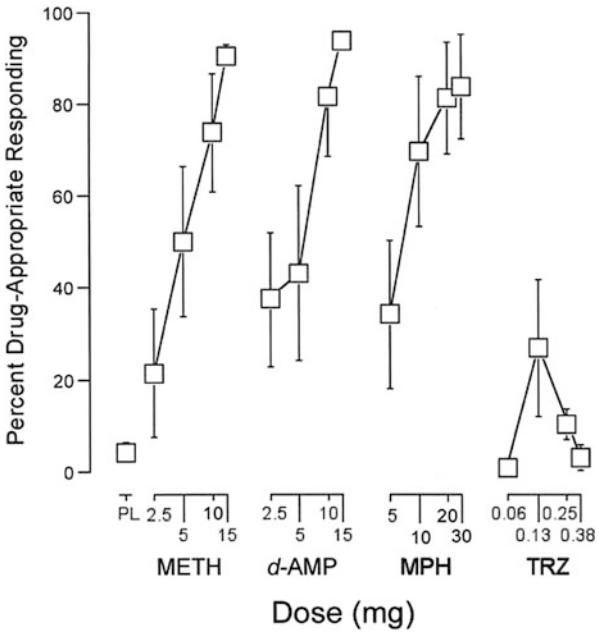

Fig. 2 Mean percent drug-appropriate responding (±SEM) during test sessions with methamphetamine (METH), D-amphetamine (D-AMP), methylphenidate (MPH), and triazolam (TRZ; negative control) in participants discriminating methamphetamine. D-Amphetamine and methylphenidate share discriminative-stimulus effects with methamphetamine but triazolam does not. *X*-axes: Test doses (mg) of methamphetamine, D-amphetamine, methylphenidate, and triazolam. Data points above PL represent values from test sessions following placebo administration. *Y*-axis: Percent drug-appropriate responding for methamphetamine. Data points represent the means of seven participants. Reprinted from Sevak et al. [17], with permission

modafinil, a NE releaser with weak affinity for the DA transporter [51, 52], nor the sedative hypnotic drug triazolam fully substituted for cocaine in this study. These findings collectively suggest that drugs that preferentially increase synaptic DA substitute for commonly abused stimulants across a range of doses; whereas, drugs that exert their primary effects through other neurotransmitter systems (e.g., triazolam and modafinil) do not produce discriminative-stimulus effects similar to commonly abused stimulants in humans.

Correspondence with Preclinical Findings The results of substitution tests in preclinical drug-discrimination studies are consistent with the notion that central monoamine systems mediate the discriminative effects of abused stimulants. For example, a range of doses of methamphetamine, cocaine, methylphenidate, D-amphetamine, and GBR 12909 were tested to determine if they shared discriminative-stimulus effects with methamphetamine in rats trained to discriminate 0.3 mg/kg methamphetamine from saline [53]. GBR 12909 is a high-affinity DA transport blocker that is considered to be selective for DA transporters [54, 55]. Each test drug substituted for methamphetamine in a dose-dependent

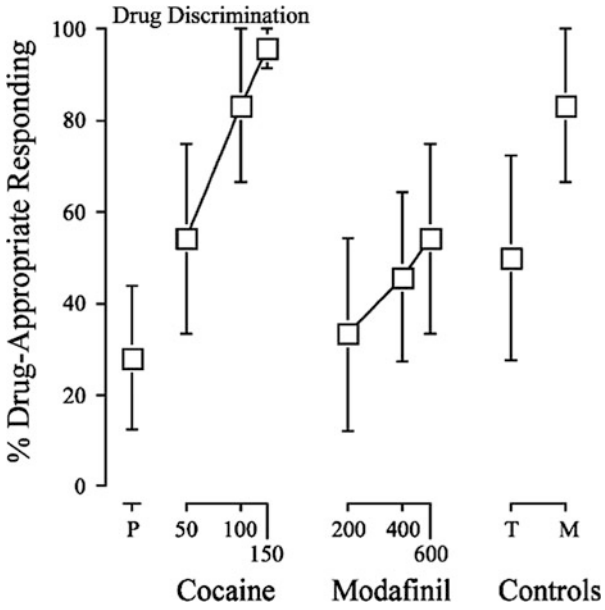

Fig. 3 Mean percent drug-appropriate responding (±SEM) for cocaine, modafinil, triazolam (T; 0.5 mg, negative control), and methylphenidate (M; 60 mg, positive control) in participants discriminating oral cocaine. Modafinil and triazolam did not substitute for cocaine suggesting they may exert their discriminative-stimulus effects through distinct neuropharmacological mechanisms. *X*-axes: Test doses (mg) of cocaine, modafinil, triazolam, and methylphenidate. Data point above P represents values from test sessions following placebo administration. *Y*-axis: Percent drug-appropriate responding for cocaine. Data points represent the means of six participants. Reprinted from Rush et al. [16], with permission

manner suggesting that DA neurotransmission contributes to the discriminative-stimulus effects of methamphetamine. Other studies have shown that DA reuptake inhibitors (e.g., bupropion, GBR 12909, and mazindol) fully substitute for cocaine whereas 5-HT and NE reuptake inhibitors do not [36, 38, 56–58]. In addition, D_1- and D_2-receptor agonists (e.g., SKF 38393 and quinpirole, respectively) engender cocaine-appropriate responding [31, 59], suggesting a prominent role for DA signaling in the discriminative-stimulus effects of abused stimulants that are concordant with the results of substitution tests in human drug-discrimination studies.

Pretreatment Studies and Underlying Neuropharmacology Although the lack of selective compounds available for use with humans limits the conclusions that may be made about the specific roles of particular monoamine systems, the results of pretreatment tests in human drug-discrimination studies also suggest that central monoamine systems mediate the discriminative-stimulus effects of commonly abused stimulants. The effects of a range of doses of D-amphetamine (i.e., 0, 2.5, 5, 10, and 15 mg), alone and following pretreatment with the D_2 receptor antagonist fluphenazine (i.e., 0, 3, and 6 mg) were assessed in participants who learned to discriminate 15 mg oral D-amphetamine from placebo [60]. Lower doses of fluphenazine (i.e., 3 mg) did not significantly alter the discriminative-stimulus effects

of D-amphetamine in this study, but a higher dose (i.e., 6 mg) produced a marked rightward shift in the D-amphetamine dose–response curve in the one participant that completed the study (Fig. 4). These findings suggest that central DA systems mediate the discriminative-stimulus effects of D-amphetamine in humans. However, these results should be interpreted cautiously because only a single subject completed the study due to the negative side-effect profile of fluphenazine.

Aripiprazole is an atypical antipsychotic that functions as a partial D_2 receptor agonist [61] and is also known to exert effects at 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_7 receptors [62]. Partial agonists can either activate receptors with decreased efficacy relative to full agonists, or conversely function as an antagonist, depending on synaptic neurotransmitter levels. To determine the effects of aripiprazole on the discriminative-stimulus effects of D-amphetamine, a range of doses of D-amphetamine (i.e., 0, 2.5, 5, 10, and 15 mg) were assessed, alone and in combination with aripiprazole (0 and 20 mg), in participants who learned to discriminate oral D-amphetamine (i.e., 15 mg) from placebo [63]. D-Amphetamine functioned as a discriminative stimulus, but aripiprazole did not engender D-amphetamine-appropriate responding when tested alone. Aripiprazole pretreatment significantly attenuated the discriminative-stimulus effects of D-amphetamine, suggesting a role for DA and 5-HT in the interoceptive effects of D-amphetamine. These results are consistent with the ability of a D_2-receptor partial agonist to function as an antagonist in the presence of a drug that elevates synaptic monoamine levels [64]. Other studies have shown similar effects with other antipsychotics and $GABA_A$ modulators such as risperidone and alprazolam, respectively [50, 65].

Pretreatment tests with agonists and antagonists in humans discriminating methamphetamine and cocaine further suggest that central monoamine systems are involved in the discriminative-stimulus effects of commonly abused stimulants (e.g., [12, 66–68] unpublished data). For example, Sevak and colleagues [66] determined the influence of aripiprazole (0 and 20 mg) on the discriminative-stimulus effects of a range of doses of methamphetamine (0, 2.5, 5, and 10 mg) in participants who had learned to discriminate 10 mg methamphetamine. Methamphetamine functioned as a discriminative stimulus and dose-dependently increased drug-appropriate responding. Aripiprazole pretreatment significantly attenuated methamphetamine-appropriate responding (Fig. 5), suggesting that monoamine systems play a role in the discriminative-stimulus effects of methamphetamine. To assess the role of monoamine systems in the discriminative-stimulus effects of cocaine, Lile and colleagues [12] tested a range of doses of oral cocaine (0, 25, 50, 100, and 200 mg) alone and in combination with aripiprazole (15 mg) in participants who had learned to discriminate 150 mg oral cocaine from placebo [12]. Although few effects of aripiprazole were observed, it appeared to attenuate the discriminative-stimulus effects of cocaine. These data collectively suggest that the discriminative-stimulus effects of commonly abused stimulants in humans are mediated by monoamine systems, namely DA and 5-HT.

Correspondence with Preclinical Findings The results of pretreatment tests in preclinical drug-discrimination studies with commonly abused stimulants correspond with those from human drug-discrimination studies and support the

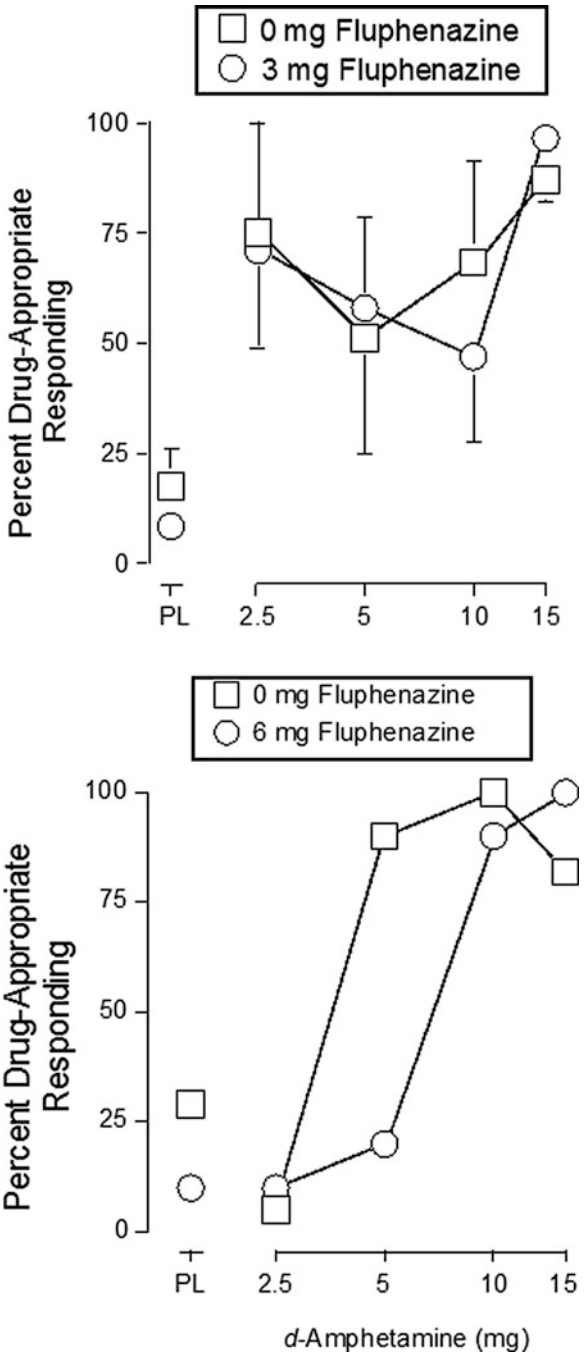

Fig. 4 Mean percent drug-appropriate responding (±SEM) following a range of doses of D-amphetamine alone and in combination with 3 mg (*circles*, *upper panel*) and 6 mg (*circles*, *lower panel*) fluphenazine. Squares represent 0 mg of fluphenazine in both panels. The 6 mg dose of fluphenazine shifted the D-amphetamine dose-effect function rightward suggesting that it attenuated the discriminative-stimulus effects of D-amphetamine. *X*-axes: D-Amphetamine dose in mg. Data points above PL represent values from test sessions following placebo administration. *Y*-axes: Percent drug-appropriate responding for D-amphetamine. Data points in the *bottom panel* represent data from one participant. Data from Stoops et al. [60]

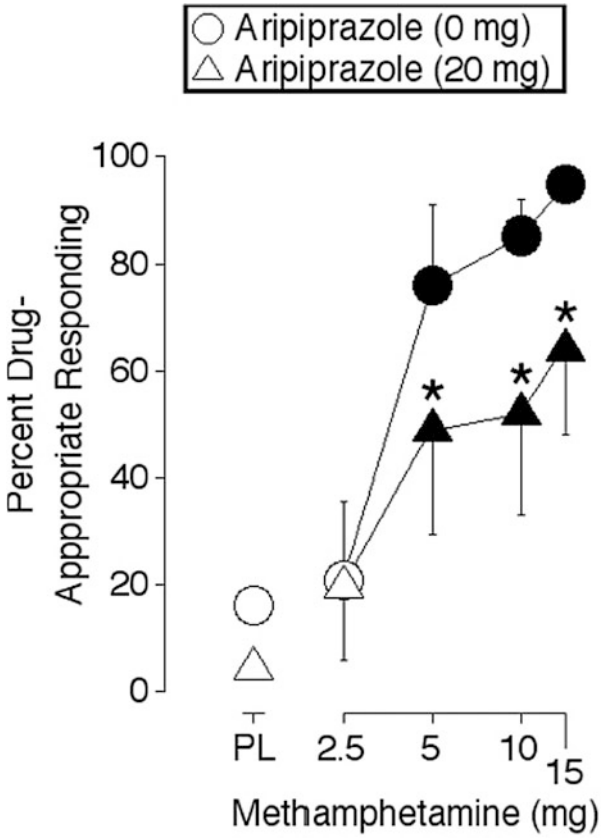

Fig. 5 Mean percent drug-appropriate responding (±SEM) during test sessions with methamphetamine alone and in combination with 0 mg (*circles*) and 20 mg (*triangles*) aripiprazole. Aripiprazole significantly attenuated the discriminative-stimulus effects of methamphetamine. *X*-axes: Methamphetamine dose in mg. Data points above PL represent values from tests with 0 mg methamphetamine (placebo) alone and in combination with 20 mg aripiprazole. *Y*-axis: Percent drug-appropriate responding. Data points represent the means of six participants. *Filled symbols* indicate a significant difference from the placebo–placebo control condition (i.e., circle above PL). An *asterisk* (*) indicate a significant difference between aripiprazole conditions at a given methamphetamine dose. Reprinted from Sevak et al. [66], with permission

hypothesis that central monoamine systems underlie the interoceptive effects of abused stimulants. For example, Mechanic and colleagues [69] determined whether the D_2 and 5-HT_2 antagonist olanzapine would attenuate the interoceptive cues elicited by D-amphetamine in rats that were trained to discriminate D-amphetamine (1.0 mg/kg) from saline. Olanzapine (1.5 mg/kg) significantly blunted the discriminative-stimulus effects of D-amphetamine. Similar findings have been obtained with selective D_1 (e.g., SCH39166) and D_2 antagonists (e.g., remoxipride and nemonapride; [70]), as well as the high-affinity dopamine transport blocker

GBR 12909 [71] to suggest a role for DA signaling in the discriminative-stimulus effects of stimulants in laboratory animals. In addition, these DA systems are under the inhibitory control of GABA systems (e.g., [72–75]). For example, Druhan and colleagues [76] showed that pretreatment with the $GABA_A$ receptor modulator midazolam (i.e., 0–0.2 mg/kg) significantly attenuated drug-appropriate responding in rats trained to discriminate D-amphetamine (i.e., 1.0 mg/kg). In sum, the results of drug-discrimination studies with humans and non-human animals suggest that the neuropharmacological mechanisms of the discriminative-stimulus effects of abused stimulants are generally consistent [77].

Summary of Drug-Discrimination Findings with Stimulants In general, data from preclinical and human drug-discrimination studies demonstrate that abused stimulants produce their interoceptive effects via activation of DA and other monoamine systems. **A**bused stimulants function as discriminative stimuli and readily substitute for one another under a wide range of laboratory conditions and across species. Drugs that share discriminative-stimulus effects with abused drugs might function as effective agonist-replacement therapies to treat stimulant-use disorders [78–81]. Alternatively, drugs that attenuate the discriminative-stimulus effects of abused drugs might function as effective pharmacotherapies for stimulant-use disorder by blunting the interoceptive effects of the drug ([82]; for a review, see [83]).

Collectively, these studies suggest that human drug-discrimination procedures are rigorous behavioral assays that may be used to elucidate the underlying neuropharmacology of the discriminative-stimulus effects of stimulants. Future studies are needed to more fully elucidate the neuropharmacological mechanisms underlying the interoceptive-stimulus effects of abused stimulants in humans. These studies might test blockers of other catecholamines or drug-combinations that may have promise as pharmacotherapies (see [83] for a review). A more comprehensive understanding of the neuropharmacological mechanisms that mediate the interoceptive effects of stimulants in humans will inform the development of putative pharmacotherapies to manage stimulant-use disorders.

2.2 Opioids

Basic Neuropharmacology and Mechanism of Action The basic neuropharmacology of opioid receptors is well known (for reviews see [84, 85]). Briefly, the mu, kappa, and delta opioid receptors belong to the class A (rhodopsin) family of $G_{i/o}$ protein-coupled receptors and are found throughout the central and peripheral nervous systems. These three receptor families mediate the analgesic effects of endogenous opioid peptides and opioid drugs [9, 85, 86]. Opioid drugs are naturally occurring, semi-synthetic, or synthetic formulations (e.g., morphine, hydromorphone, and fentanyl, respectively). They are further classified as full agonists, partial or mixed agonists/antagonists, and full antagonists based on their pharmacological actions, selectivity, affinity and efficacy at the three primary

receptor families [9]. The majority of prescribed opioid analgesics are agonists at the mu receptor with relatively limited activity at the other receptor types. The abuse-related behavioral effects of prototypical opioids like morphine, heroin, or hydromorphone have largely been attributed to their interaction with the mu receptor family [87–89]. The mu receptor family, in particular, is known to modulate the neuropharmacological activity of monoamine and GABAergic neurotransmitter systems resulting in increased synaptic dopamine levels [90–92]. The kappa and delta opioid receptor families are structurally and functionally similar to mu opioid receptors [85]. However, the behavioral effects of drugs that activate kappa and delta opioid receptors differ from those that preferentially activate mu receptors. For example, kappa agonists can produce dysphoria and hallucinations and there is evidence that the kappa receptor family is involved in stress responses [93]. Delta receptor agonists are less susceptible to analgesic tolerance compared to mu receptor agonists suggesting that these receptors may produce analgesic effects via different pharmacological mechanisms [94]. This section of the chapter focuses on the mu receptor because most opioids that have been tested affect mu activity and the mu receptor is most clinically relevant with regard to opioid dependence in humans.

Substitution Profile Eleven published clinical studies have examined the discriminative-stimulus effects of opioid drugs [14, 95–104]. A seminal study by Preston and Bigelow [98] illustrates that opioid agonists with similar efficacy and affinity for the mu receptor generalize other mu receptor agonists but do not generalize opioid agonists that differ in these respects. Volunteers with a history of regular opioid use learned to discriminate intramuscular saline, hydromorphone, and butorphanol using the three-choice discrimination procedure (i.e., Drug A, Drug B, or Drug C) to investigate the discriminative-stimulus effects of hydromorphone and other opioid drugs with varying degrees of affinity for mu and kappa opioid receptors. The opioid drugs that were tested included hydromorphone (0.375–3.0 mg), the partial mu and kappa receptor agonist pentazocine (7.5–60 mg), the mu and kappa receptor mixed agonist–antagonist butorphanol (0.75–6 mg), the non-selective opioid agonist nalbuphine (3.0–24 mg), and the partial mu receptor agonist buprenorphine (0.075–0.6 mg). Opioids with greater affinity for the mu receptor fully substituted for hydromorphone regardless of whether the drug was a partial or full agonist. Opioids with lower intrinsic activity at mu receptors did not substitute for the mu agonist hydromorphone. Figure 6 shows that hydromorphone occasioned dose-related increases in hydromorphone-appropriate responding but did not substitute for butorphanol, consistent with their hypothesized neuropharmacological actions at the mu opioid receptor.

Correspondence with Preclinical Findings Preclinical research with pigeons [105, 106], rats [107–111], and non-human primates [112, 113] have consistently shown that the discriminative-stimulus effects of opioids are concordant across species and that these effects follow with their in vitro neuropharmacology. For example, Platt and colleagues [113] investigated the discriminative-stimulus effects

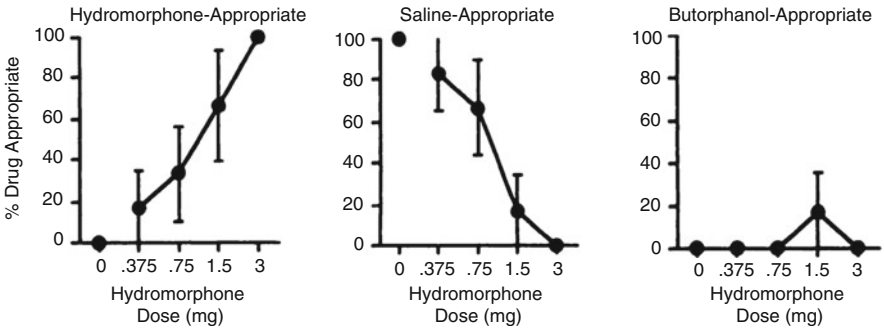

Fig. 6 Mean percent drug-appropriate responding (±SEM) during substitution tests with hydromorphone in participants discriminating hydromorphone, saline, and butorphanol. Hydromorphone significantly increased hydromorphone-appropriate responding but did not substitute for any dose of butorphanol tested. The discriminative-stimulus effects of mu-opioid receptor agonists follow their predicted neuropharmacological actions. *X*-axes: Hydromorphone dose in mg. *Y*-axes: Percent drug-appropriate responding for hydromorphone (*left*), saline (*center*), and butorphanol (*right*). Data points represent the means of six participants. Reprinted from Preston and Bigelow [98], with permission

of heroin in non-human primates and showed that the interoceptive effects of heroin were largely attributable to mu opioid receptor activation. Substitution tests with the major metabolites of heroin (i.e., 6-monoacetylmorphine, morphine, morphine-6-glucuronide, and morphine-3-glucuronide) and the mu opioid receptor agonists fentanyl and methadone were conducted with rhesus monkeys trained to discriminate heroin from saline. Each of these drugs occasioned dose-dependent increases in heroin-appropriate responding and, on average, engendered full substitution for heroin.

Pretreatment Tests and Underlying Neuropharmacology We know of two published clinical studies that have used pretreatment strategies to investigate the discriminative-stimulus effects of opioid drugs [104, 114]. For example, Strickland and colleagues [104] utilized antagonist pretreatment in conjunction with substitution strategies to demonstrate that some of the discriminative-stimulus effects of the atypical opioid tramadol are mediated by mu receptor activation. Figure 7 shows representative drug-discrimination data for two subjects following administration of hydromorphone or a range of doses of tramadol alone (circles) or in combination with 50 mg naltrexone (squares). Tramadol occasioned dose-related increases in drug-appropriate responding for tramadol and a test dose of hydromorphone occasioned partial or full substitution for tramadol. Pretreatment with naltrexone (50 mg, p.o.) significantly attenuated the discriminative-stimulus effects of tramadol and hydromorphone. The use of opioid antagonists in human drug-discrimination procedures is an important strategy that provides additional information about the underlying neuropharmacological mechanisms of opioid drugs. Further, the use of this strategy bridges preclinical and clinical research; thereby, strengthening the translational validity of findings from drug-discrimination studies. Unfortunately, there are few clinical studies that have used antagonist

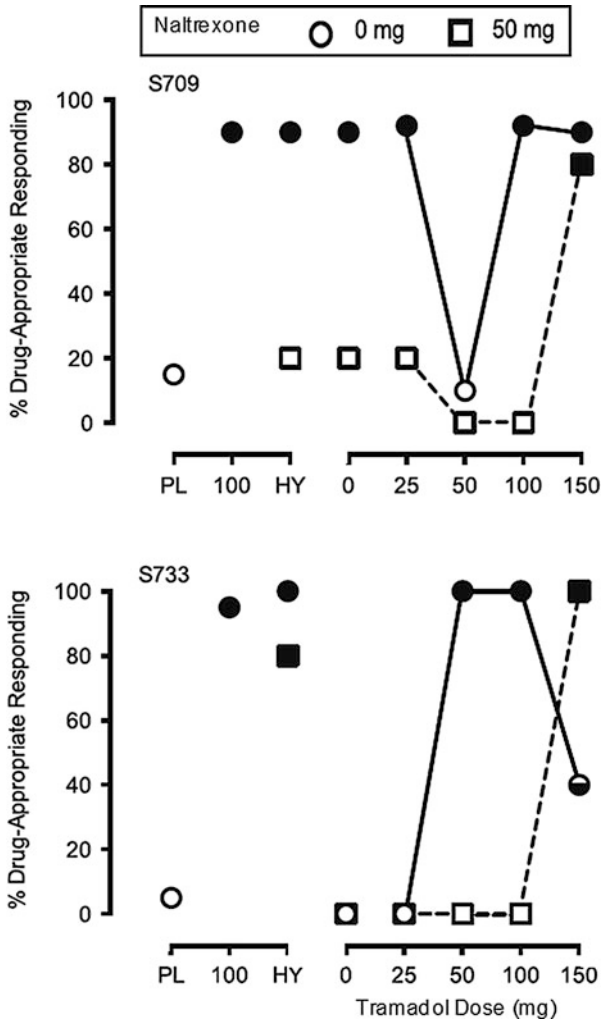

Fig. 7 Percent drug-appropriate responding from two subjects following administration of 4 mg hydromorphone (HY) and a range of doses of oral tramadol alone and in combination with 0 mg (*circles*) and 50 mg (*squares*) naltrexone. Hydromorphone substituted for tramadol in both subjects and naltrexone attenuated the tramadol discriminative stimulus in one subject. Tramadol increased drug-appropriate responding at several doses. Naltrexone attenuated these effects at lower tramadol doses. These findings suggest that mu-opioid receptors are at least partly involved in the discriminative-stimulus effects of tramadol. *X*-axes: Tramadol dose in mg. Data points above PL and 100 represent values from test of acquisition sessions following administration of placebo and 100 mg tramadol, respectively. *Filled symbols* indicate full tramadol substitution (i.e., $\geq$80% tramadol-appropriate responding). *Half-filled shapes* indicate partial substitution (i.e., 21–79% tramadol-appropriate responding). *Y*-axes: Percent drug-appropriate responding for tramadol. Reprinted from Strickland et al. [104], with permission

pretreatment procedures to elucidate the neuropharmacological underpinnings of the discriminative-stimulus effects of opioids.

Correspondence with Preclinical Findings Preclinical work using pretreatment strategies has been crucial for examining the neuropharmacology of the discriminative-stimulus effects of opioid drugs. For example, France and colleagues [115] trained pigeons to discriminate morphine from placebo and then performed substitution tests with morphine and oxymorphazone (a mu opioid receptor agonist). Morphine and oxymorphazone occasioned morphine-appropriate responding in a dose-dependent manner. Pretreatment with naltrexone shifted the dose–response curves to the right, indicating that naltrexone attenuated the discriminative-stimulus effects of these drugs. Antagonism of the discriminative-stimulus effects of opioid drugs by naltrexone pretreatment has also been observed in rhesus monkeys that were trained to discriminate heroin or morphine from vehicle [112, 113, 116].

Summary of Drug-Discrimination Findings with Opioids Opioid drug-discrimination studies in both human and non-human animals using substitution and pretreatment procedures are remarkably consistent with their neuropharmacological binding profiles for the mu receptor. These studies have revealed that although the discriminative-stimulus effects of opioid drugs are not limited to activity at opioid receptors, they are primarily mediated by mu receptor activity. These results are consistent with a primary role for the mu receptor in the ability of repeated opioid administration and dosing cessation to induce dependence and withdrawal, respectively (reviewed in [117]). This neuropharmacological overlap in clinically relevant effects suggests that opioid drug-discrimination procedures could be used for medications development [19]. Opioid drugs with decreased abuse potential that share discriminative-stimulus effects with abused opioids might be effective pharmacotherapies for opioid dependence.

2.3 Δ^9-Tetrahydrocannabinol (Δ^9-THC)

Basic Neuropharmacology and Mechanism of Action Of the more than 60 cannabinoid compounds found in cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC) is widely considered to be primarily responsible for its psychoactive effects [118]. The behavioral effects of Δ^9-THC are mediated through the endogenous cannabinoid neurotransmitter system, which is composed of two known receptor subtypes: CB_1 and CB_2 [119, 120]. Both cannabinoid receptor subtypes are G-protein-coupled receptors that inhibit adenylate cyclase activity and activate mitogen-activated protein kinase, but they differ to some degree in their interactions with certain ion channels and other G-proteins (e.g., [121–123]). CB_1 and CB_2 receptors also differ in their distribution such that CB_1 receptors are primarily expressed on presynaptic nerve terminals throughout the central and peripheral nervous systems; whereas, CB_2 receptors are expressed on immune cells

[123]. Although Δ^9-THC is a non-selective partial agonist at CB_1 and CB_2 receptors, at least four lines of evidence suggest that the central effects of Δ^9-THC are primarily mediated through CB_1 receptors. First, the in vivo potency of Δ^9-THC correlates with its binding affinity at the CB_1 receptor [124]. Second, the CB_1 receptor subtype is localized in areas of the central nervous system that correspond with Δ^9-THC effects [125]. Third, agonists that are selective for CB_1 receptors produce behavioral effects more similar to Δ^9-THC than selective CB_2 agonists [126–128]. Lastly, the centrally mediated effects of Δ^9-THC are blocked by the administration of CB_1-selective antagonists, but not those selective for CB_2 receptors [129–132]. Given that another principal function of cannabinoid receptors is the modulation of non-cannabinoid neurotransmitter release via retrograde signaling [133], other neurotransmitter systems also likely play a role in the behavioral effects of cannabinoids.

The published literature concerning the discriminative-stimulus effects of Δ^9-THC in humans is much smaller in comparison to the other drug classes discussed in this chapter. To the best of our knowledge, only 8 studies have been published that evaluated the discriminative-stimulus effects of Δ^9-THC in humans [134–141]. In more recent studies, participants learned to discriminate orally administered Δ^9-THC versus placebo. The use of orally administered Δ^9-THC in lieu of smoked cannabis improves pharmacological selectivity (as cannabis contains other cannabinoids), allows better control of dosing parameters, and eliminates peripheral cues associated with smoked cannabis (e.g., [134]). The available literature on the discriminative-stimulus effects of orally administered Δ^9-THC and its underlying neuropharmacology as determined with human drug-discrimination procedures is reviewed below.

Substitution Profile The substitution of other drugs for the discriminative-stimulus effects of Δ^9-THC in humans has been determined in several studies [135–140]. However, most of these studies determined the effects of a test drug alone (i.e., substitution) and in combination (i.e., pretreatment) with Δ^9-THC (i.e., [136–139]). The results of pretreatment tests are discussed below in a separate section for ease of comparison. In the first study by Lile and colleagues [140], eight cannabis users learned to discriminate 25 mg oral Δ^9-THC versus placebo. After learning the discrimination, a range of oral doses of Δ^9-THC (5–25 mg), triazolam (0.0675–0.375 mg), hydromorphone (0.75–4.5 mg), and methylphenidate (5–30 mg) was substituted for the training dose. Figure 8 shows that oral Δ^9-THC engendered dose-related increases in drug-appropriate responding, whereas none of the other drugs occasioned significant Δ^9-THC-like responding. Worth mentioning is that each of the drugs tested produced measurable effects on other study outcomes, confirming that biologically relevant doses were tested. Lile and colleagues [140] determined the substitution profile of the mixed CB receptor agonist nabilone in six human cannabis users who learned to discriminate 25 mg Δ^9-THC from placebo. As shown in Fig. 9, nabilone dose-dependently substituted for the interoceptive-stimulus effects of Δ^9-THC with the highest doses of nabilone (3 and 5 mg) fully substituting for the training dose. In contrast, methylphenidate

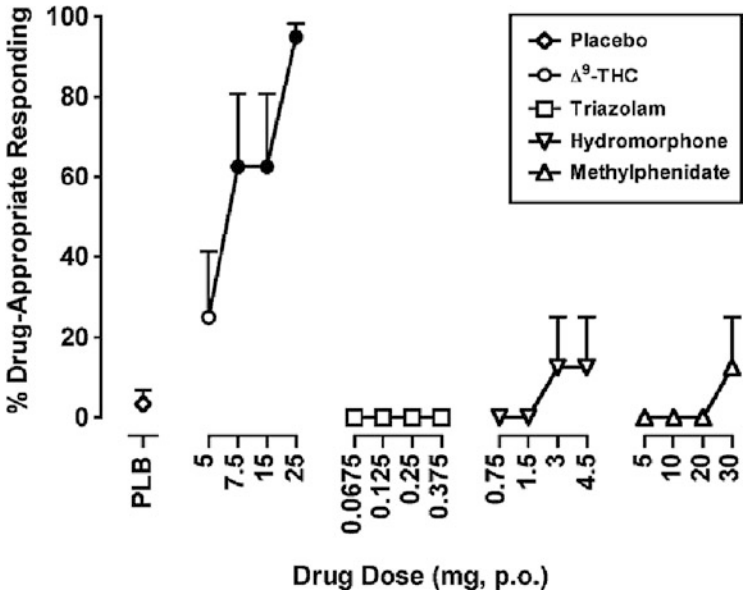

Fig. 8 Mean percent drug-appropriate responding (±SEM) during test sessions with Δ^9-THC (*circles*), triazolam (*squares*), hydromorphone (*inverted triangles*), and methylphenidate (*triangles*) in humans discriminating oral Δ^9-THC. Δ^9-THC functioned as a discriminative stimulus and its discriminative-stimulus effects are not directly mediated by other central neurotransmitter systems. *X*-axes: Oral drug dose in mg per os. The data point (*diamond*) above PLB represents data following placebo administration. All data points represent the means of eight participants. *Filled symbols* indicate a significant difference from placebo (PLB). *Y*-axis: Percent drug-appropriate responding for Δ^9-THC. Reprinted from Lile et al. [140], with permission

did not significantly increase drug-appropriate responding, similar to a previous study [140]. These findings demonstrate the pharmacological selectivity of the discriminative-stimulus effects of Δ^9-THC and suggest that cannabinoid receptors are central to the Δ^9-THC discriminative stimulus but other receptor systems (e.g., GABA) are not.

Correspondence with Preclinical Findings The results of substitution tests with human subjects discriminating Δ^9-THC are relatively consistent with the results of non-human animal studies. Specifically, cannabinoid agonists occasion drug-appropriate responding in animals discriminating Δ^9-THC (e.g., [126, 131, 142–144]), but mu-opioid agonists (e.g., heroin and morphine) generally do not share discriminative-stimulus effects with Δ^9-THC in animals [127, 131, 145–149]. Preclinical studies have also shown that dopaminergic drugs generally do not substitute for the discriminative-stimulus effects of Δ^9-THC [127, 131, 150]. However, the results with triazolam and diazepam in humans [139, 140] do not agree with the preclinical findings that positive modulators of the $GABA_A$ receptor partially substitute for the discriminative-stimulus effects of Δ^9-THC [145, 146, 149, 151–153].

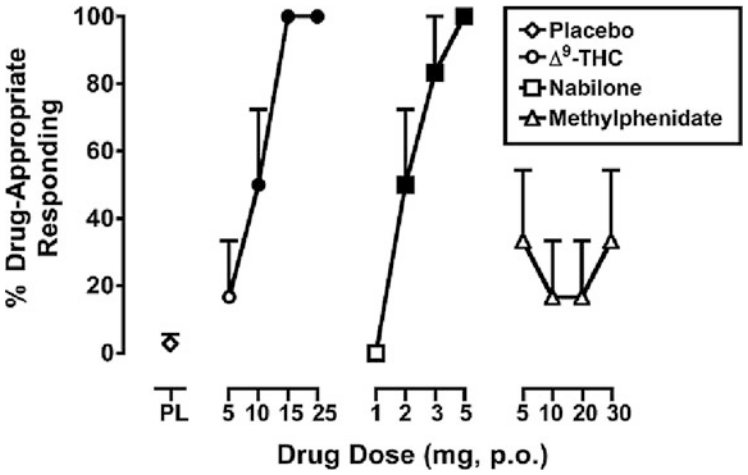

Fig. 9 Mean percent drug-appropriate responding (±SEM) during test sessions with Δ^9-THC (*circles*), nabilone (*squares*), and methylphenidate (*triangles*; negative control) in humans discriminating oral Δ^9-THC. Nabilone dose-dependently substituted for Δ^9-THC suggesting that the discriminative-stimulus effects of Δ^9-THC and nabilone are primarily mediated by activation of the cannabinoid receptor system. *X*-axes: Oral drug dose in mg per os. The data point (*diamond*) above PL represents data following placebo administration. *Y*-axis: Percent drug-appropriate responding for Δ^9-THC. Data points represent the means of six participants. Error bars were omitted on certain data points for clarity. Reprinted from Lile et al. [135], with permission

Pretreatment Tests and Underlying Neuropharmacology Five studies have used drug-discrimination procedures to investigate the underlying neuropharmacology of the Δ^9-THC discriminative stimulus in humans [136–139, 141]. These studies used similar procedures to determine the role of the cannabinoid and GABA neurotransmitter systems in the discriminative-stimulus effects of Δ^9-THC. Briefly, participants in these studies learned to discriminate 30 mg of oral Δ^9-THC versus placebo in a two-choice (i.e., Drug vs. Not Drug) procedure. During testing, participants received three doses of nabilone (0, 1, and 3 mg p.o.), tiagabine (0, 6, and 12 mg p.o.), diazepam (0, 5, and 10 mg p.o.), and baclofen (0, 25, and 50 mg p.o.) alone and in combination with oral Δ^9-THC (5, 15, and 30 mg). Figure 10 shows that nabilone occasioned Δ^9-THC-appropriate responding when administered alone and shifted the Δ^9-THC dose-effect function upward and leftward when co-administered with Δ^9-THC [136]. Similarly, the GABA reuptake inhibitor tiagabine fully substituted for the Δ^9-THC discriminative stimulus at the highest dose tested (12 mg) when administered alone and shifted the Δ^9-THC dose–response curve upward and leftward in a dose-related manner [137]. In subsequent studies, the $GABA_A$ positive modulator diazepam did not occasion Δ^9-THC-like responding when administered alone, in agreement with earlier triazolam results [140], and did not systematically affect the discriminative-stimulus effects of Δ^9-THC when administered in combination [139]. In contrast, a high dose of the $GABA_B$ agonist baclofen (50 mg) partially substituted for the Δ^9-THC discriminative stimulus and both doses

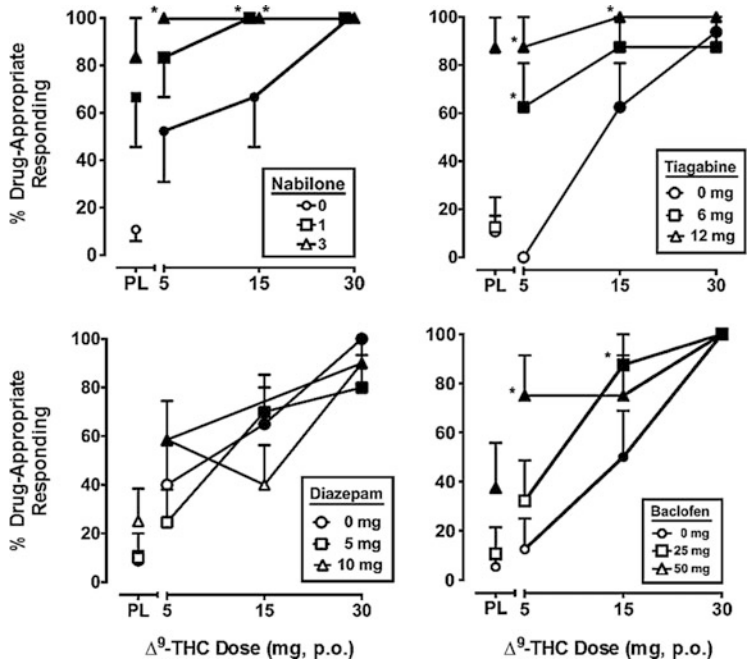

Fig. 10 Mean percent drug-appropriate responding (±SEM) following Δ^9-THC (5, 15, and 30 mg), alone and in combination with three doses of nabilone (*upper left*), tiagabine (*upper right*), diazepam (*bottom left*) and baclofen (*bottom right*) in humans discriminating oral Δ^9-THC. These findings suggest the involvement of cannabinoid and $GABA_B$ neurotransmitter systems in the discriminative-stimulus effects of Δ^9-THC in humans. *X*-axes: Oral drug dose in mg per os. Data points above PL represent data for each test drug dose following 0 mg Δ^9-THC. *Y*-axes: Percent drug-appropriate responding for Δ^9-THC. For nabilone, tiagabine, diazepam, and baclofen, data points represent the means of six, eight, eight, and ten participants, respectively. In all panels, *filled symbols* indicate a significant difference from placebo–placebo (circle above PL). *Asterisks* (*) indicate a significant difference from a given dose of oral Δ^9-THC alone (circles in each panel). Error bars were omitted in certain instances for clarity. Reprinted from Lile et al. [136–139], with permission

of baclofen significantly enhanced Δ^9-THC-appropriate responding when co-administered [138]. These findings collectively demonstrate the involvement of $GABA_B$ receptor subtype, in the discriminative-stimulus effects of Δ^9-THC in humans.

Correspondence with Preclinical Findings Procedural differences preclude the direct comparison of preclinical and human laboratory studies because most preclinical studies have determined the effects of pretreatment with cannabinoid antagonists on the discriminative-stimulus effects of Δ^9-THC instead of cannabinoid agonists or GABA ligands. For example, pretreatment with the cannabinoid receptor antagonist rimonabant attenuates the discriminative-stimulus effects of Δ^9-THC in laboratory animals (e.g., [143, 154–156]). Despite these differences, some

consistent findings emerge. First, drugs that activate the cannabinoid receptor system engender Δ^9-THC-appropriate responding in humans and animals supporting the assertion that the cannabinoid receptor system is critically involved in the discriminative-stimulus effects of Δ^9-THC (e.g., [126, 131, 135, 136, 142–144]). Second, stimulation of GABA neurotransmission appears to play a role in the discriminative-stimulus effects of Δ^9-THC in both humans and preclinical animal models but the mechanisms that mediate these effects may differ between species [137–139, 145, 146, 149, 151–153].

Summary of Drug-Discrimination Findings with Δ^9-THC Although the body of research that has examined the underlying neuropharmacology of Δ^9-THC in human subjects is relatively small, the extant literature demonstrates that cannabinoid and GABA neurotransmitter systems are important contributors to the discriminative-stimulus effects of Δ^9-THC in humans. However, there appear to be species differences in the GABA-specific receptor mechanisms between humans and non-human animals. Lastly, the activation of monoamine (e.g., DA) and mu-opioid receptors does not appear to be involved in the interoceptive effects of Δ^9-THC in humans. These studies also provide insight into potential therapeutic targets for the treatment of cannabis-use disorders. More specifically, these findings suggest that GABA could be targeted in the development of medications for cannabis dependence. In fact, gabapentin, a GABA analog that is approved for treating neuropathic pain and seizures, has recently emerged as a promising candidate pharmacotherapy for cannabis-use disorder [157] and, to date, is the only medication that has demonstrated initial pharmacotherapeutic efficacy in clinical trials in adults. Future research is needed to disentangle the mechanism by which gabapentin reduces cannabis use and also to determine whether a GABA reuptake inhibitor or $GABA_B$ agonist would be useful for managing cannabis dependence. In sum, drug-discrimination studies have greatly enhanced our understanding of the underlying neuropharmacology of Δ^9-THC in humans and have helped to identify potential neuropharmacological targets for the treatment of cannabis dependence.

2.4 General Summary

This section reviewed a number of studies that used human drug-discrimination techniques to investigate the underlying neuropharmacology of stimulants, opioids, and the primary psychoactive constituent in cannabis, Δ^9-THC. At least four overarching conclusions can be drawn from the drug-discrimination literature reviewed above: (1) drugs in each of these classes function as discriminative stimuli in humans, (2) the discriminative-stimulus effects of these drugs are generally consistent with their underlying neuropharmacology, (3) the discriminative-stimulus effects of drugs in these classes are conserved across species, and (4) drug-discrimination techniques allow the determination of the underlying neuropharmacology of commonly abused illicit drugs to identify potential therapeutic

targets that may guide the development and evaluation of putative pharmacotherapies for substance-use disorders.

3 Current State and Future of Human Drug-Discrimination Research

The primary objective of this chapter was to provide a basic procedural overview of human drug-discrimination procedures and summarize the extant literature regarding the underlying neuropharmacology of commonly abused drugs (i.e., stimulants, opioids, and cannabis) as determined via human drug-discrimination studies. Although the extant literature firmly establishes human drug discrimination as a highly versatile and useful behavioral assay of in vivo neuropharmacology, interest in human drug-discrimination research and drug-discrimination research in general, has waned somewhat since its peak in the late 1990s. One factor that has potentially led to the decrease in enthusiasm for drug-discrimination studies in substance-abuse research is that the role of discriminative-stimulus effects in substance abuse may be less apparent relative to behavioral processes that are the focus of other experimental approaches. McMahon [19] articulates a particularly poignant example when addressing the downward trend in the publication of drug-discrimination compared with the continued increase in the publication of drug self-administration research. Specifically, he cites that drug discrimination lacks the strong face validity of drug self-administration with regard to substance abuse because operant behavior maintained by a drug reinforcer more closely resembles the behavioral phenomenon of substance abuse [19]. Although behavioral models that have high face validity are intuitively appealing, whether or not they effectively predict the outcome of a manipulation on the phenomenon that they are intended to model is more important. The validity of the drug-discrimination paradigm for identifying the underlying neuropharmacology of centrally acting drugs in whole organisms is virtually unparalleled. However, less research has centered on the role that the discriminative-stimulus effects of drugs play in substance abuse but they may play a particularly important role in relapse and the resumption of problematic drug use.

Although the use of human drug-discrimination procedures in the future is uncertain, the emergence and growing popularity of designer drugs (i.e., bath salts), synthetic marijuana (i.e., spice), and devices that are used to vaporize nicotine (e.g., e-cigarettes) and cannabis will create new opportunities for additional drug-discrimination research. Furthermore, creative thinking about the application of human and laboratory animal drug-discrimination procedures to the investigation of interoceptive events that may contribute to substance abuse (e.g., drug withdrawal, anxiety, stress, etc.) may also provide opportunities for the use of these procedures to investigate the abuse-related behavioral effects of drugs in addition to underlying neuropharmacology.

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Conditioned Taste Avoidance Drug Discrimination Procedure: Assessments and Applications

Anthony L. Riley, Matthew M. Clasen, and Mary A. Friar

Abstract In the present chapter, we summarize much of the work on the taste avoidance drug discrimination procedure, presenting the logic for its initial introduction and the extension of the procedure in the investigation of the discriminative properties of various drugs. Results from these assessments parallel those from more traditional operant and maze designs in classifying and characterizing the discriminative properties of drug. At the same time, this design reveals a procedure that is sensitive in such assessments by indexing these stimulus properties more rapidly and at lower doses than in the more traditional procedures (in some cases for drugs heretofore resistant in their detection). Importantly, much remains to be learned about the taste avoidance procedure in that the nature of such learning remains unknown and the specific parameters under which it can be established and generalized and its neurochemical and neuroanatomical bases are largely unexplored. The application of drug discrimination learning to human drug abuse continues to be an important consideration for this specific design (as well as that of drug discrimination procedures in general), and recent parallels between drug use and food intake in terms of its regulation by interoceptive stimuli suggests a possible role of the loss of stimulus control in drug escalation and addiction (with possible therapeutic implications via the modulation of these interoceptive cues).

Keywords Conditioned taste avoidance • Drug discrimination learning • Drug classification and characterization • Drug use and abuse

A.L. Riley (✉), M.M. Clasen, and M.A. Friar
Psychopharmacology Laboratory, Center for Behavioral Neuroscience, American University, Washington, DC 20016, USA
e-mail: alriley@american.edu

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Contents

1 Introduction

Although operant and maze designs have been used primarily in assessing the stimulus properties of drugs (see Overton and Porter, this volume), Riley and his colleagues (see [1]; see also [2–4]) described a relatively unique preparation that utilized classical conditioning in the assessment of a drug's subjective effects, specifically, conditioned taste avoidance learning (see below). The logic for the use of this procedure was based on early work by John Garcia and his colleagues on the tendency of rats to avoid consuming various foods that had been paired or associated with toxicosis. Specifically, Garcia et al. [5] reported that rats that had been irradiated following consumption of a novel saccharin solution decreased consumption of the solution on subsequent exposures. The avoidance was presumably based on the rat's association of the sweet saccharin solution with the aversive effects of the radiation. This avoidance was rapidly acquired (often in a single pairing; [5]), very robust (to the point of complete suppression of consumption; [6]), occurred despite long delays between consumption of the solution and the onset of radiation [7] and was relatively selective to taste, i.e., environmental cues paired with the radiation were not readily associated with its effects [7]. Garcia and his colleagues argued that these characteristics of taste avoidance learning reflected evolutionary pressures on mammals that contributed to their avoidance of poisoned foods (for reviews, see [8, 9]).

Although taste avoidance learning was initially discussed and assessed for its relatively unique characteristics (all of which challenged traditional learning theory; see [9, 10]), subsequent work with the preparation focused on the various conditions under which it was established and expressed, e.g., other drugs, species, tastes, temporal parameters (see [10]), its physiological, biochemical and molecular underpinnings (see [11]) and relatively recently its translational application to a variety of clinical and behavioral issues (for reviews, see [10, 12]). In relation to the latter point, taste avoidance learning was applied to the suppression of predation in wild animals, the control and treatment of alcohol abuse, the elimination or reduction of cancer-induced anorexia, the nature and control of immunosuppression, and the assessment of the biochemical mediation of learning and memory.

2 Taste Avoidance Drug Discrimination Procedure

Given the relatively unique ability of animals to rapidly and robustly associate taste with illness, using a drug to signal this pairing might allow the development of a rapid assay of the stimulus effects of drugs. Specifically, if an animal is given a drug prior to the pairing of a taste and some aversive agent and the drug vehicle prior to a presentation of saccharin alone, it might learn the signaling function of the drug. Under such conditions, the animal would come to avoid the taste when it was preceded by the drug (that signaled the taste-toxin pairing) and consume the same taste when it was preceded by an injection of the drug vehicle. That is, the animal would learn the drug discrimination within the taste avoidance preparation. The function of the drug in this preparation is functionally identical to that seen in the more traditional drug discrimination procedures, i.e., the drug signals some programmed contingency. Again, the difference is that the general taste avoidance design is so rapidly acquired and robust that the taste avoidance procedure may provide an efficient and effective assay of drug discrimination learning.

In this vein, my laboratory tested this prediction with the glutamate channel blocker phencyclidine (PCP; see [1]). In this assessment, adult female Long-Evans rats were adapted to a restricted water schedule (20-min day) until consumption stabilized. They were then given a novel saccharin solution for 3 habituation days during which a vehicle injection was given 10 min prior to each saccharin access. On the 1st drug day, subjects in Group PL were given an injection of PCP (1.8 mg/kg) 10 min prior to access to the saccharin solution (for 20 min). This saccharin access was then followed immediately by an injection of the toxin LiCl (1.8 mEq). For this group, P stood for the PCP pretreatment; L stood for administration of LiCl following saccharin consumption. Subjects in a second group (Group PW) were also injected with PCP prior to the 20-min saccharin access but they were injected with the LiCl vehicle immediately following its consumption. For this group, P again stood for the PCP pretreatment; W stood for administration of the LiCl vehicle following saccharin consumption. On the following 3 days, subjects in both groups were given the PCP vehicle prior to access to saccharin which was followed by the LiCl vehicle, i.e., 3 safe days. This alternating procedure was repeated until all subjects had received five complete cycles. Following the fifth cycle, the above-mentioned procedure was repeated except that on the 2nd recovery day of each cycle various doses of PCP, ketamine, and D-amphetamine were given prior to saccharin access to assess their ability to substitute for PCP. On these probe sessions, saccharin access was followed by the LiCl vehicle (see Fig. 1 for a schematic of the general procedures used in this design).

As expected from work with PCP in more traditional assessments of drug discrimination learning (see [13, 14]), the animals learned the PCP/vehicle discrimination. Specifically, subjects in Group PL avoided the saccharin solution when it was preceded by the injection of PCP and consumed the same saccharin solution when it was preceded by an injection of the PCP vehicle. Importantly, and consistent with our earlier predictions, the discrimination was acquired very rapidly. In

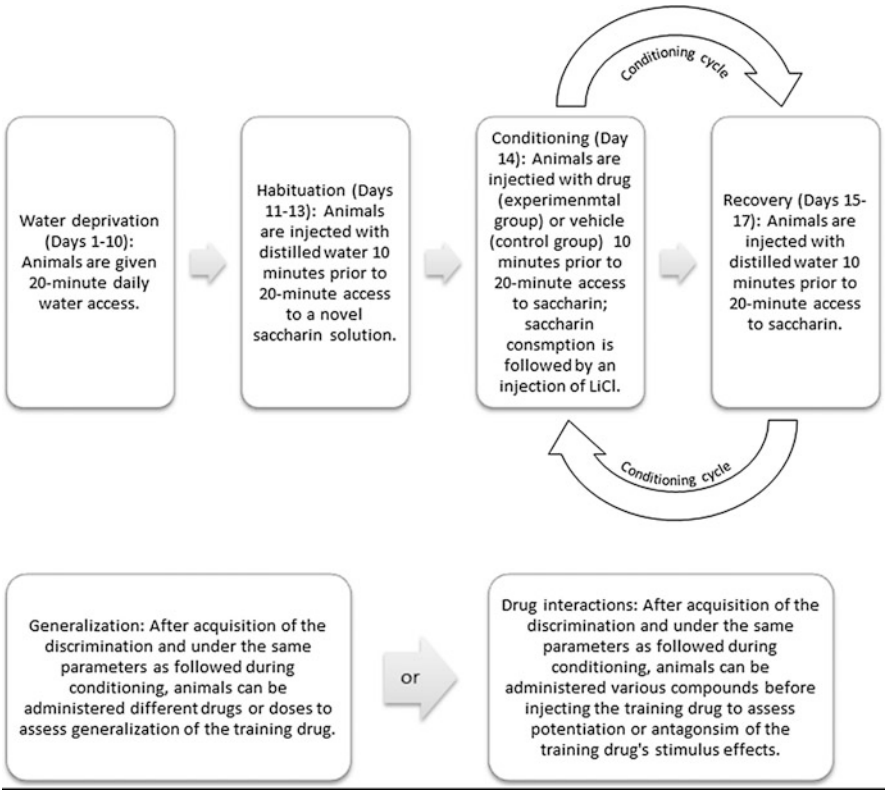

Fig. 1 Schematic diagram depicting the stages and timeline of the conditioned taste avoidance drug discrimination procedure (*top panel*). Once the discrimination has been acquired, slight modifications in the procedure allow for the assessment of drugs to substitute, potentiate or block the stimulus effects of the training drug by administering other compounds on test days given on the second recovery session of the conditioning cycle (*bottom panel*)

fact, after only two conditioning cycles (8 days total) subjects in Group PL drank significantly less saccharin on the PCP treatment days than on the vehicle treatment days (see Fig. 2). That this difference did not reflect any unconditioned effects of PCP on fluid consumption was evident in the fact that subjects in Group PW, those injected with PCP prior to a saccharin-vehicle pairing, drank saccharin at high levels following both PCP and vehicle pretreatment days, and saccharin consumption on these days did not differ. Clearly, PCP came to serve a discriminative function, in this case that either saccharin was aversive or that saccharin (or drinking) was paired with LiCl (see below). On subsequent probe sessions, different doses of PCP (1, 1.8, and 3.2 mg/kg) substituted for the training dose (1.8 m/kg), with the greatest substitution occurring at the highest dose. The PCP-like compound ketamine dose-dependently substituted for PCP, while the psychostimulant D-amphetamine did not (see Fig. 3).

In this same study, a different group of animals (Group WL) was given the PCP vehicle prior to the saccharin-LiCl pairing and PCP prior to a pairing of saccharin

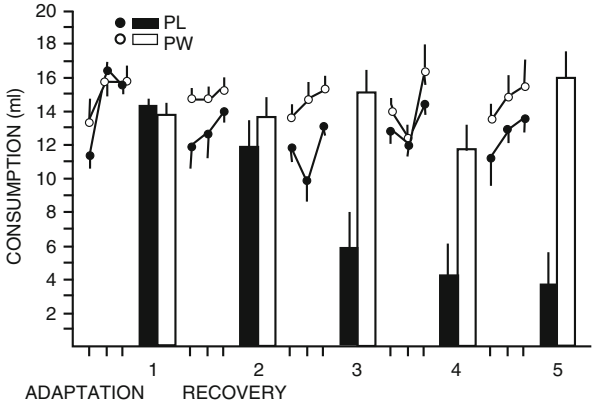

Fig. 2 Mean absolute saccharin consumption for subjects in Groups PL and PW during adaptation and throughout the repeated conditioning and recovery cycles (see text for details). From Mastropaolo et al. [1]

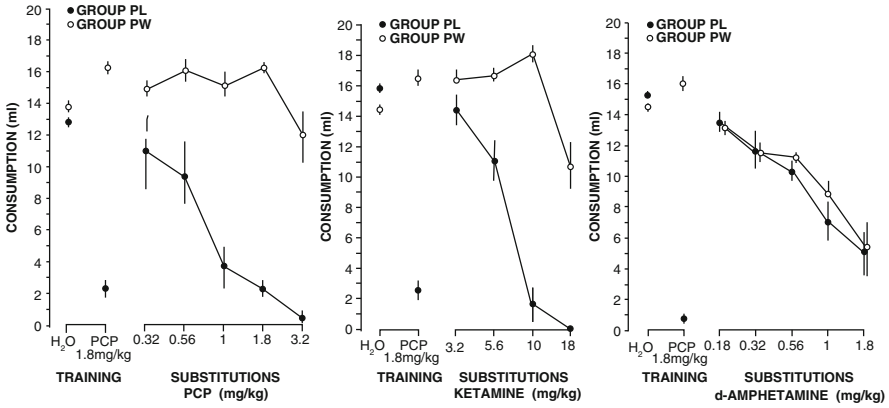

Fig. 3 Mean absolute saccharin consumption for subjects in Groups PL and PW during training and when given various does of PCP (*left panel*), ketamine (*middle panel*) and D-amphetamine (*right panel*) during multiple generalization tests following conditioning (see text for details). Figure redrawn from Mastropaolo et al. [1]

with the LiCl vehicle (for a fourth group, Group WW, both the PCP vehicle and PCP signaled a pairing of saccharin with the LiCl vehicle). This procedure was utilized to test whether the drug had to signal a taste-toxin pairing to be effective as a discriminative cue. Under these conditions, the discrimination was again rapidly learned (after two conditioning cycles), except here animals in Group WL avoided consumption of saccharin when access was preceded by the vehicle injection and consumed the saccharin when it was preceded by PCP. Control subjects in Group WW drank saccharin at high levels following both the vehicle and PCP (see Fig. 4). During substitution probes, consumption was suppressed at low doses of PCP and gradually increased as the probe dose approached the training dose. Similar

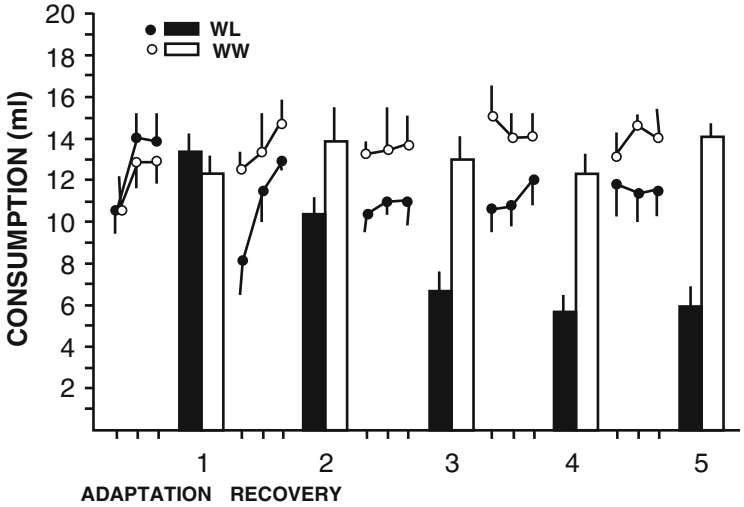

Fig. 4 Mean absolute saccharin consumption for subjects in Groups WL and WW during adaptation and throughout the repeated conditioning and recovery cycles (see text for details). From Mastropaolo et al. [1]

dose–response functions were evident with ketamine. Amphetamine suppressed consumption at every dose, suggesting that it was discriminated from PCP and treated as the vehicle.

It was clear from our initial assessment that a drug effective as a discriminative stimulus in more traditional drug discrimination designs served effectively as a discriminative stimulus in the conditioned taste avoidance procedure we use in our laboratory. Interestingly, discriminative control under the conditioned taste avoidance procedure was achieved with a lower dose (1.8 mg/kg compared to 4.0 mg/kg) than the previously published literature exploring the stimulus properties of PCP [14]. Further, the rate of acquisition of the discrimination was more rapid in the taste avoidance procedure than typically reported in more traditional assessments. Importantly, the rate and patterns of substitution did not appear to be dependent upon whether the drug signaled a taste-toxin or a taste-vehicle pairing (though see below). What was important was that the drug signaled some behavioral contingency.

Concurrent with our work with PCP in this preparation, others reported similar drug discrimination learning using the taste avoidance procedure. Lucki [3], for example, demonstrated the procedure's ability to characterize the specific receptor systems mediating the discriminative effects of the serotonergic agonists 8-OH-DPAT (5-HT_{1A}) and TFMPT ($5HT_{1B/1C}$) (see also [15]; [16–19]). Martin and his colleagues ([4]; see also [20]) attempted to characterize the nature of the learning occurring in the taste avoidance procedure and reported that the animals undergoing discrimination training with morphine had not learned anything specific about the taste-toxin pairing but instead had learned that morphine signaled

something about a class of responses, e.g., drinking, and its consequences (see Mastropaolo et al. for a similar suggestion with PCP; see also [21–23]). Jaeger and Mucha [2] added one final twist to the initial findings with the taste avoidance drug discrimination procedure by demonstrating that while the procedure was a sensitive index of discriminative control (with the acquisition of the discrimination rapid under training conditions for both pentobarbital and fentanyl), the control established, the generalization produced, and the drug substitutions seen were a function of the training drug and the training procedures, i.e., drug danger vs. drug safe; see above. (For a description of an alternative model using the taste avoidance procedure, specifically the cross-familiarization design, see [24–27]; [28].)

3 Sensitivity of the Taste Avoidance Procedure

These first four studies established the taste avoidance procedure to assess the discriminative stimulus effects of drugs (and displayed the procedure's ability to classify and characterize the drug stimulus), initiated assessments into the specific receptor mechanisms mediating the stimulus effects of a number of drugs, addressed the nature of such learning, and illustrated differences in discriminative control as a function of the training drug and training conditions. What followed from these initial reports was a series of studies that extended these initial investigations to other drugs and to other conditions under which such learning could occur. Several studies also addressed the neurochemical and neuroanatomical substrates of such learning via specific agonist probes and antagonist challenges [19, 29] and selective chemical placement and lesions (see [20, 30, 31]). In relation to the specific training drug, a wide variety of compounds, e.g., acetaldehyde [32], alprazolam [33], amphetamine [34, 35], buprenorphine [36, 37], cocaine [38], chlordiazepoxide [39–41], D-amphetamine [42, 43], diprenorphine [44], ethanol [24, 45, 46], fluvoxamine [27], indorenate [16–19], morphine [4, 20, 21, 30, 31, 47–54], nalorphine [37], naloxone [55, 56], tetrahydrocannabinol (THC) [57], and rimonabant [58], have now been assessed for their ability to establish discriminative control. Although discriminative control can be established to each of these drugs, there was another feature of these assessments that characterized the taste avoidance procedure, i.e., control was rapidly acquired and to a degree often significantly faster than the control seen under the more traditional operant procedures, arguing that the taste avoidance design is a sensitive index of such learning. Further, for several drugs, e.g., cholecystokinin [29, 59–61], estradiol [62], naloxone [63], and testosterone ([64]; for a comprehensive table describing each study which utilizes the CTA procedure of DDL contact, alriley@american.edu), discriminative control was established at lower doses than required in operant assessments. That is, the taste avoidance procedure was not only more sensitive in the speed with which discriminations were acquired, but also in terms of the dose needed to acquire the discrimination.

One example of such a drug is the opioid antagonist naloxone. Although naloxone had been reported to serve as a discriminative cue in opiate-dependent animals (via precipitated withdrawal; [65]), it was generally ineffective as such a cue unless high doses were used or animals were subjected to extended training. For example, in one of the first attempts at establishing naloxone stimulus control in morphine-naïve rats, Colpaert et al. [66] reported that naloxone (at a dose range of 10–160 mg/kg) failed to serve as a discriminative cue in an operant procedure in which food served as the reinforcer (see also [65, 67]). Similarly, Overton and Batta [68] reported that the majority of rats in a shock-escape T-maze procedure failed to reach criterion performance at a dose of 25 mg/kg naloxone, even after 60 training sessions (see [69]). Interestingly, although Carter and Leander [70] reported that pigeons could acquire a discrimination based on naloxone in a food-reinforced design, these effects were only at 30 mg/kg and after an average of 79 sessions, again documenting the relatively weak stimulus effects of naloxone. In this context, Kautz and her colleagues [63] attempted to establish naloxone discriminative control using the taste avoidance procedure. The logic for this attempt paralleled that used above with other assessments within this procedure, i.e., taste avoidance learning is robust and rapidly acquired and thus may provide a more sensitive index of drug discrimination learning. Using this procedure, Kautz et al. injected rats with 1 or 3 mg/kg naloxone 10 min prior to a pairing of a saccharin solution with the emetic LiCl (1.8 mEq, 0.15 M; 76 mg/kg). On subsequent recovery days, the animals were given an injection of the naloxone vehicle prior to a pairing of saccharin with the LiCl vehicle. The conditioning cycle was repeated until the discrimination was acquired. Control subjects were injected with naloxone prior to saccharin consumption as well, but saccharin was never followed by LiCl for this group. As with PCP (and other compounds previously tested in this procedure), the naloxone discrimination (at both doses) was rapidly acquired (in this case by the third conditioning cycle – 12 consecutive days) with subjects injected with naloxone drinking significantly less saccharin than controls (see Fig. 5). The fact that controls did not show the same suppression indicated that the suppression of consumption by the conditioned subjects was not a function of any unconditioned effects of naloxone. Subsequent generalization tests revealed that the relative selective mu opioid antagonist naltrexone substituted completely for naloxone (and at lower doses than the training drug); whereas, the mu agonist morphine did not. This work revealed that opioid antagonists (like opioid agonists) could serve as discriminative stimuli in rats and that this discriminative control could be established at low doses and after only a few training trials. Although the basis for the discriminative control was not assessed in the Kautz et al. work, it was likely that naloxone's discriminative effects are a function of antagonism of endogenous opioid tone. Independent of the specific basis of these effects, the discriminative effects of naloxone were likely mediated at the mu receptor subtypes of the opioid receptor given that animals trained to discriminate naloxone (1 mg/kg) from its vehicle in the taste avoidance procedure generalize control to the relatively selective mu antagonist naltrexone, but not to the selective delta antagonist, naltrindole, nor the selective kappa antagonist, MR2266 (Fig. 6; see [55]; for other assessments

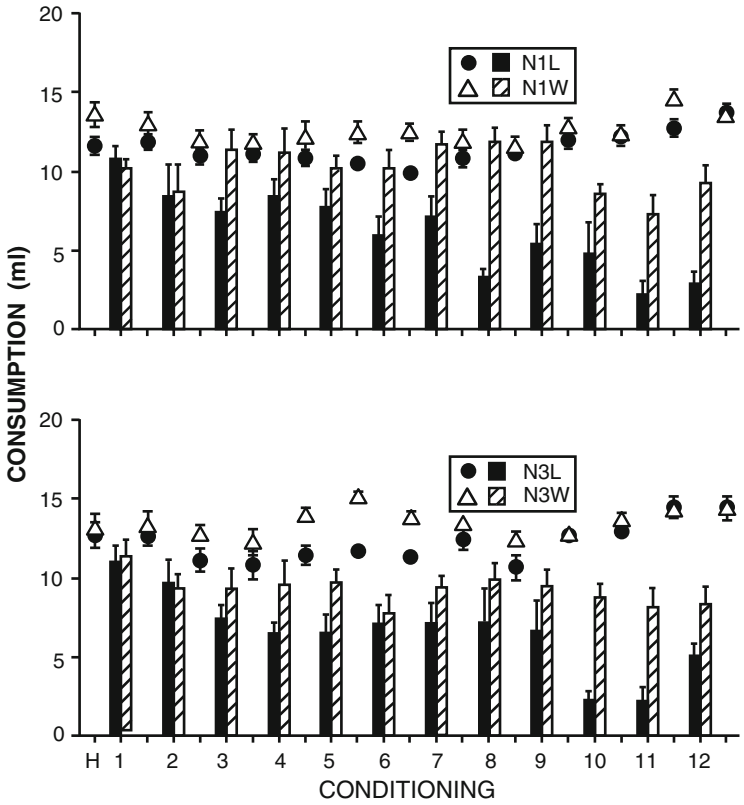

Fig. 5 Mean absolute saccharin consumption for subjects in Groups NL and NW during adaptation and throughout the repeated conditioning and recovery cycles (see text for details). Subjects were trained with either 1 (N1; *upper panel*) or 3 (N3; *lower panel*) mg/kg of naloxone. From Kautz et al. [63]

of opiate antagonist and mixed agonist/antagonist discriminative control, see [36, 37, 44, 56, 71, 72]).

The speed with which drug discrimination is acquired and the dose required for such acquisition relative to the traditional operant design have been used by us and others to argue that taste avoidance may be a more sensitive index of such learning [2–4, 15, 73]. While suggestive, it is important to note several caveats on this position. First, although the criteria used to index discriminative control is generally well defined for operant procedures, e.g., 80% drug-appropriate responding following the drug [74], there are no established criteria used in the taste avoidance procedure. Individual researchers have indicated stimulus control when consumption following the drug is significantly different than that following the vehicle (for a discussion of this issue, see [45]) or when consumption in the conditioned group following the drug is significantly different than that in the control group following the drug. While each of these comparisons in the taste avoidance procedure indexes

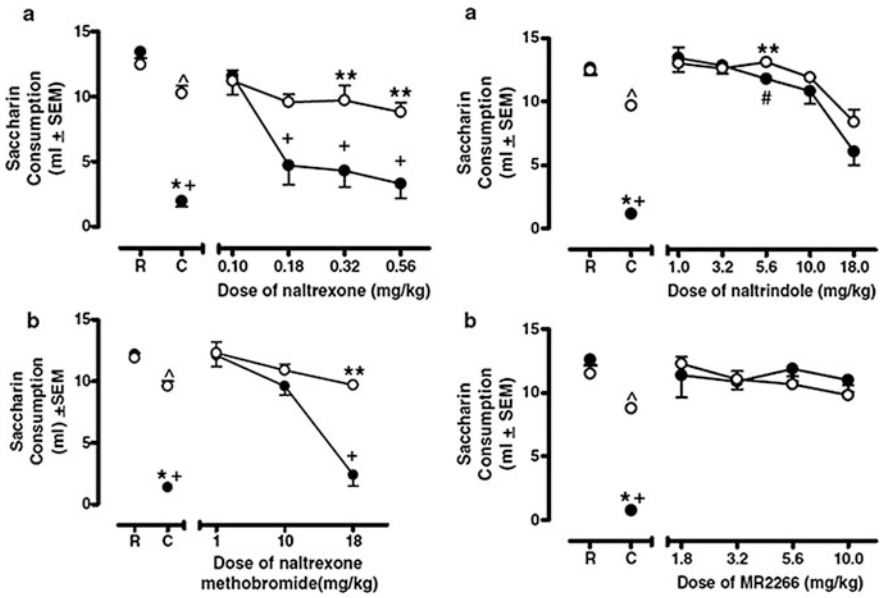

Fig. 6 Mean absolute saccharin consumption for subjects in Groups NL (*closed circles*) and NW (*open circles*) during conditioning (C) and recovery (R) and when given various does of naltrexone (*upper left panel*), naltrindole (*upper right panel*), naltrexone methobromide (*lower left panel*), and MR2266 (*lower right panel*) during multiple generalization tests following conditioning. Figure redrawn from Davis et al. [55]

the fact that discrimination has been acquired, it is not clear at all that this is a comparable measure to that used in the operant designs. As such, comparative statements about relative sensitivity must be cautiously made.

Secondly, the parameters used in the taste avoidance and operant procedures are not always comparable. In fact, most comparisons between the two designs are generally made across studies and across laboratories (for an exception, see [26]). It is crucial that direct comparisons be made in the speed of acquisition and the dose sufficient to establish control. Only then can relative statements about sensitivity and additional comparisons in terms of the drugs that can substitute for or block discriminative control, species, sex and age differences, and neurochemical and neuroanatomical substrates be confidently made. It is interesting to note that what initially appeared to be significant differences in the processing of the drug stimulus in the operant and taste avoidance designs (i.e., graded or quantal; for a discussion, see [75–80]) eventually was reported to be similar when the two procedures were assessed under comparable parametric procedures (see [72]).

Finally, although stimulus control within the taste avoidance procedure is generally discussed as being consistent across all parametric analysis, there are differences dependent upon the training drug and training conditions, indicating that its relative sensitivity may be dependent upon these factors (see [2]). It is interesting in this context that although discriminative control with estradiol is

difficult to demonstrate in traditional operant procedures (see above), Gorzalka et al. [81] were able to establish control with estradiol in a Y-maze shock-escape task. Such comparisons suggest that relative sensitivity may be evident in other designs as well and is likely a function of the specific nature and conditions of the procedures used to train and assess stimulus control.

4 Caveats

As described above, the conditioned taste avoidance drug discrimination procedure appears to be able to classify and characterize the discriminative properties of drugs in a manner similar to that seen in more traditional operant and maze assessments (although few comparisons have been directly made between the various designs). Importantly, such discriminative effects are more rapidly acquired and are evident at lower doses in the taste avoidance procedure. There are several caveats with the procedure, however, which need to be addressed (for a discussion, see [40, 82]). One issue raised about this procedure is the necessity of multiple groups when assessing the stimulus properties of any drug. As described above, in any general assessment of the discriminative effects of a given drug in the avoidance procedure, the animal is injected with a drug prior to a pairing of a fluid with an injection of an aversion-inducing agent and injected with the drug vehicle prior to consumption of the same fluid but not followed by the aversive agent. If the drug used as the discriminative cue unconditionally suppresses consumption, e.g., has unconditional adispsogenic effects, the suppression seen following the drug may be a result not of its cueing function but instead its unconditioned effects on fluid consumption. Because of this, control subjects are generally tested that receive the drug prior to fluid access alone (but not paired with an emetic-like LiCl) on both conditioning and recovery days. These subjects serve as a control for the effects of the drug on fluid intake and provide a comparison with the conditioned subjects to substantiate the cueing function of the drug in this group. In operant designs, the animal serves as its own control, injected with the drug prior to the responding on one lever for food and the drug vehicle prior to responding on another lever for the same reinforcer. Given that the measured response is an active lever or key press, one can immediately see if the drug unconditionally suppresses response rate. In the taste avoidance design, suppression of consumption is the measured response and has to be controlled by this second group. Consequently, the number of animals used in the taste avoidance procedure is doubled.

Although this increase in the number of subjects is somewhat offset by the fact that multiple animals can be concurrently run with little increase in time (or equipment), others have addressed the potential confounding issue of unconditioned drug-induced suppression of fluid consumption by other procedures that do not necessarily require additional subjects. For example, following discrimination training in which an animal is injected with a stimulus drug prior to the pairing of a taste with a toxin (and on recovery days, injected with vehicle prior to the taste

alone), one can give the animal access to both the taste and water in a two-bottle test of the ability of the stimulus drug to suppress consumption (see [3, 4, 15–17, 19, 34, 35, 42, 83]). If the drug is simply suppressing consumption in general, both saccharin and water intake would be affected; if the drug suppresses consumption by virtue of its signaling function, only that taste would be affected and the taste would be selectively avoided relative to water which is what generally is reported under such assessments.

Others have addressed this need for a control for the unconditionally suppressive effects of the drug by injecting the animal with the drug prior to a taste paired with the toxin vehicle, i.e., as a safety cue. In this design (see above), animals are injected with the drug vehicle prior to the pairing of the taste with the toxin and injected with the drug prior to the presentation of the taste alone [1, 2, 4, 19, 52]. Animals readily acquire the drug discrimination, avoiding saccharin when it is preceded by vehicle and consuming the same saccharin when preceded by the drug (see above, Fig. 4). If the drug has unconditioned suppressive effects on fluid consumption, the discrimination would be impaired (as animals decrease consumption of the taste preceded by the drug). As noted, under these conditions, animals readily acquire the discrimination with a variety of drugs, e.g., pentobarbital, morphine, PCP, and indorenate.

Although several caveats have been discussed in relation to the taste avoidance drug discrimination procedure, it remains highly effective as a design in detecting the stimulus effects of a variety of drugs and to and does so at low doses and relatively rapidly. The extent to which the procedure is effective in behavioral pharmacology and generally used will be weighed against its possible limitations (and adaptations to circumvent potential confounds).

5 Drugs as Interoceptive Stimuli for Regulated Drug Intake

Drug discrimination learning in general is an effective tool in the classification and characterization of drugs (see [84, 85]). In so doing, the procedure can identify and class a drug according to the stimulus properties it shares with other drugs, e.g., opioids, stimulants, and depressants. It can also be used to identify the biological and neurochemical substrates of drug action as other pharmacological probes potentiate or antagonize the drug's stimulus effects, yielding information on the specific receptor systems mediating the drug's effects and the efficacy of the drug at a specific receptor (e.g., as a partial or full agonist). The model has gone beyond these basic pharmacological assessments to its use in clinical pharmacology as a procedure to identify drugs that induce or abate anxiety or drugs that induce dependence and produce withdrawal (see [86]). One area in which the basic drug discrimination procedure has been extended (and often with controversy; see [87]) is in its assessment of the abuse liability of various drugs. The use of the design in

this vein stems from the fact that drugs of abuse are used by humans for their subjective effects, and if the drug discrimination procedure is a reliable and robust index of such effects it may be useful in corroborating or predicting use, abuse and dependence. Although often described in this context, there is little evidence of its utility in this area, and several arguments and demonstrations have been made against its general use as such a predictor. For example, although most drugs of abuse can serve as discriminative stimuli, other drugs, many with no abuse liability, serve such a function as well [84]. Further, when direct assessments have been made between abuse liability and degree of discriminability, there was no relationship (see [68]).

Importantly, most investigators in drug discrimination research recognize the limitation of this design in indexing abuse vulnerability and argue instead that the procedure is simply one additional tool in assessments of drug abuse and one that should be used in conjunction with other assays (ones often with more face validity, e.g., self-administration) to index the rewarding and addicting properties of drugs (see [74]). Although drug discrimination learning may be limited in its capacity to identify abuse vulnerability (at least in the manner by which it is typically used; see above), Panlilio et al. [88] have presented an interesting model that may, nonetheless, have importance to drug use and abuse. Specifically, Panlilio et al. argue that drug taking (as indexed in animal self-administration models) displays highly regular patterns of responding and pauses. They argue that the initiation and maintenance of responding is a function of the drug's rewarding effects. The pauses are indicative of the fact that the drug is either not rewarding or aversive. That is, animals initiate responding when the drug level is low (or absent) and responding at these times is highly rewarding to the animal. Immediate responding after this initial bout is not rewarding given that the system mediating the drug's rewarding effect is saturated. With normal metabolic processes, the drug levels are reduced below some trigger or set point such that an infusion at this point is again rewarding. The issue is how does the animal know what the blood level is to induce or suppress responding? Given the well-established phenomenon that drugs do have subjective effects in animals (as assessed by the drug discrimination procedure), Panlilio et al. argue that these effects serve as cues to when a behavioral response (e.g., bar press) will or will not be reinforced. In this view, regulated drug intake is monitored and controlled by the discriminative effects of the drug itself (see also [89–92]).

This view on the patterning of drug self-administration parallels work by Davidson and his colleagues examining regulatory food intake (for a review, see [93]). They have argued that the initiation of food intake is based heavily on food cues that drive consumption. Like regulated drug intake, animals also regulate the amount consumed. That is, following a bout of eating, animals stop food intake, although the very same cues present at its initiation may still be available. Regulation of feeding, according to Davidson comes from satiety cues that inhibit further eating. These cues work to inform the animal that continued eating is either no longer reinforcing or is aversive. This hypothesis necessitates the ability of the animal to detect such cues, and in direct assessments of this hypothesis (using a

drug discrimination learning design), he and his colleagues have shown that animals can use deprivation (satiety) cues to control behavior. For example, in one such experiment (see [94]) animals were given a 1 ma foot shock for 0.5 s when 24 h deprived but not when 0 h deprived (another group had these contingencies reversed such that they were shocked when 0 h deprived and not shocked under the 24 h deprivation condition). The amount of time spent freezing was used to index learning for both groups. Although both groups displayed more freezing than a non-shocked control group, the level of freezing was a function of the deprivation level, i.e., those shocked under the 24 h condition froze more when 24 h deprived than when 0 h deprived, whereas those shocked under the 0 h deprivation condition froze more when 0 h deprived than when 24 h deprived, indicative of the ability of the deprivation states to serve as discriminative cues for responding (see also [95, 96]; see [97] for similar assessments in a food-motivated task).

The parallels between eating and drug taking are interesting for other reasons beyond a potentially common process, i.e., discriminative control by interoceptive stimuli. Another parallel is that both types of behaviors can be dysregulated. In the case of eating, such dysregulated behavior can lead to excessive eating and obesity. In the case of drug use, dysregulated behavior can lead to escalated drug intake and addiction. Interestingly, a model proposed by Davidson and his group suggests the basis for this dysregulation may be similar between the two conditions (see [98, 99]). Specifically, Davidson and his colleagues have shown that animals with hippocampal damage have difficulty in behavioral tasks dependent upon inhibitory control, e.g., serial feature negative discriminations in which one cue signals some outcome but not when that cue is preceded by another stimulus [100]. In this case, the second stimulus informs the animal that the original stimulus-outcome contingency is not available and the animal inhibits responding in the presence of this feature negative stimulus (negative occasion setter). Animals with hippocampal lesions have deficits in displaying this inhibition and consequently have deficits in serial feature negative tasks [101]. Davidson and his group has further demonstrated that animals exposed chronically to high fat, western diets have loss of integrity of the blood–brain-barrier relatively selective to the hippocampus and also display deficits in hippocampal mediated task, e.g., serial feature negative discriminations [93, 102, 103]. The relevance to obesity comes from the fact that such animals (those exposed to high fat diets) may be displaying overeating and obesity as a function of the hippocampal damage that reduces the animal's ability to inhibit responding to food-related cues, i.e., they can't use these interoceptive cues to inform them that food is no longer rewarding and to inhibit feeding. Thus, in this model, excessive food intake and obesity are a consequence of high fat diet exposure that impacts discrimination learning or its expression.

Although little has been done in the context of drug abuse in this model, it is interesting and important to note that both acute and chronic cocaine (as well as methamphetamine and morphine) impairs blood–brain-barrier integrity (see [104–106]) and impairs performance on serial feature negative tasks (see [107]). Chronic drug use (as use goes from impulsive to compulsive that is characteristic of addiction; see [108, 109]) has been suggested to be a function of a host of

neuroplastic changes including downregulation of brain reward pathways and upregulation or recruitment of brain stress systems that shift basic drug use from its positively to its negatively reinforcing effects (see [110]). If there are also drug-induced changes in BBB permeability that allow for the movement of cytokines and glia into the brain (and selectively into the hippocampus) that result in hippocampal damage and the loss of inhibitory control, changes in drug intake (as already demonstrated for food intake) may also be mediated by these neuroplastic changes. Traditionally, this latter position of reduced inhibitory control has been thought to be regulated by the cortex (dorsolateral, orbitofrontal, and anterior cingulate cortex), but as the parallels between dysregulated food intake and drug intake show, this deficit may also be a function of the loss of discriminative control of regulated drug intake due to selective hippocampal damage (which may be mediated via its interconnections with the prefrontal cortex; see [111, 112]). More research will be needed to assess the role of the hippocampus and discriminative control in drug abuse and addiction, as well as the ability of the taste avoidance drug discrimination procedure to detect differences in drug-naïve and drug-exposed animals. It is interesting in this context that the specific taste avoidance drug discrimination procedure in which the drug signals the safe presentation of a taste (and the drug vehicle signals the taste-toxin pairing; see above) is an example of negative occasion setting or feature negative learning. The taste avoidance drug discrimination procedure should provide an additional model to assess the effects of drugs on BBB integrity, hippocampal function, and behavioral deficits, expanding the use of this model to understanding the potentially important role that drug discrimination learning may play in both regulated and dysregulated behavior in general and human drug abuse and addiction, specifically.

6 Conclusions

Work with the taste avoidance drug discrimination procedure has revealed a preparation that is both rapid and sensitive in its ability to index the discriminative stimulus properties of drugs. The design further allows for assessments of the ability of other drugs to substitute for the training drug (providing an ability to classify compounds with shared stimulus effects) and for manipulations that block and/or potentiate its stimulus control (providing a procedure to characterize the neurochemical basis and neuroanatomical locus of the training drug's stimulus effects). Work with this procedure parallels the effects reported in other designs, although the speed of acquiring stimulus control and the sensitivity in its detection may make this a useful behavioral tool in such assessments. Further, although a host of drugs have been examined in the taste avoidance drug discrimination procedure, much remains to be done to determine the conditions under which stimulus control is established, the degree to which this control is dependent upon specific parameters, the nature of the learning within this design, its neurochemical and neuroanatomical mediation and its limitations. The investigation of the role of drug

discrimination learning in both normal and dysregulated behavior in humans continues to be an important consideration, and recent work with cognitive deficits (and obesity) as well as drug use and abuse illustrates the potential utility of drug discrimination learning in understanding the basis for these behaviors. As such, the experimental assessment and clinical applications of drug discrimination learning (within both traditional operant and the taste avoidance drug discrimination procedures) may continue to provide insights to basic pharmacology and translational opportunities for the understanding and treatment of human pathology.

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A Prospective Evaluation of Drug Discrimination in Pharmacology

Ellen A. Walker

Abstract As investigators, we use many methodologies to answer both practical and theoretical questions in our field. Occasionally, we must stop and collect the latest findings or trends and then look forward to where our ideas, findings, and hypotheses may take us. Similar to volumes that were published in previous years on drug discrimination (Glennon and Young, Drug discrimination applications to medicinal chemistry and drug studies. Wiley, Hoboken, 2011; Ho et al., Drug discrimination and state dependent learning. Academic Press, New York, 1978), this collection in Current Topics in Behavioral Neurosciences serves as a current analysis of the continued value of the drug discrimination procedure to the fields of pharmacology, neuroscience, and psychology and as a stepping stone to where drug discrimination methodology can be applied next, in both a practical and theoretical sense. This final chapter represents one investigator's perspective on the utility and possibilities for a methodology that she fell in love with over 30 years ago.

Keywords Abuse liability testing · Complex cues · Drug discrimination · Interoceptive states · Receptor theory

Contents

For several decades, drug discrimination has been used as a tool to understand the pharmacology of different drug classes or has been involved in the discovery of new drug targets or receptors (Porter et al. 2018). This trend continues today. In a practical sense, drug discrimination is an excellent procedure to understand the

E. A. Walker (✉)
Department of Pharmaceutical Sciences, Temple University School of Pharmacy, Philadelphia, PA, USA
e-mail: ellen.walker@temple.edu

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underlying pharmacology, mechanisms, and functional outcomes for drug-receptor interactions. Bar none, the pharmacological selectivity, orderly adherence to biological principles, and sensitivity to antagonism made drug discrimination a key tool in neuropharmacology. As stated by the late Francis Colpaert "... the DD [*drug discrimination*] paradigm offers an exquisitely specific, selective, and sensitive approach to the in vivo analysis of drug-receptor interactions ..." (Colpaert 2011).

1 Drug Discrimination as a Tool to Define Receptor Pharmacology

In the current volume, a number of excellent chapters have reviewed the history and our current understanding of drug-receptor interactions as defined by drug discrimination methodology for a range of drug classes in various species, including humans. For example, Mori and Suzuki (2016) nicely outlined the necessity for 5-HT_2 receptor activation as the critical component with a clear role for 5-HT_{1A} modulatory function for the discriminative stimulus effects of hallucinogens such as MDMA and LSD. Furthermore, through drug discrimination, investigators were able to differentiate the contributions of 5-HT to the effects of MDMA and distinguish substitution patterns for different psychostimulants such as *N,N*-DMT, 5-MeO-DMT, and methamphetamine. These patterns could then be compared and contrasted to cocaine and opioid discriminative stimuli (Mori and Suzuki 2016). In the opioid field, the high selectivity of opioid drug discrimination is readily demonstrated as only MOP, KOP, or DOP receptor ligands substitute for morphine, U50,488, SNC80, and BW373U86 discriminative stimuli and only receptor selective antagonists such as CTAP, nor-BNI, or naltrindole will block these cues, respectively (Butelman and Kreek 2016). More recently, drug discrimination has been extended to the selectivity of NOP or nociceptin receptor ligands. For example, when the NOP receptor agonist Ro 64-6198 was trained as a discriminative stimulus in rats, morphine, U50,488, and SNC80 failed to substitute for Ro 64-6198 and Ro 64-6198 failed to substitute for morphine in rats trained to discriminate morphine suggesting this NOP receptor agonist is selective for NOP and no other opioid receptors (Recker and Higgins 2004). Finally, a classic collection of studies on drug discrimination in receptor classification was reviewed by Rosecrans and Young (2017). In these studies, investigators demonstrated that the (−)-nicotine discriminative stimulus was blocked by antagonists such as mecamylamine and DHβE (dihydro-β-erythroidine), which demonstrated the roles of α4β2 nicotinic acetylcholine receptors in the brain and for underlying the discriminative stimulus effects of (−)-nicotine.

An additional requirement in the classification of drug-receptor interactions and the understanding of pharmacological action is the demonstration of stereoselectivity, sensitivity to time course, and pharmacokinetics to substitution patterns. For example, the stereoselectivity or time course for opioids (Butelman and Kreek 2016) and

stimulants (Berquist and Fantegrossi 2017; Rosecrans and Young 2017) has long played an important role in determining patterns of stimulus substitution and discriminability for different training drugs. Interestingly, Negus and Banks (2016) actually use the relationship of pharmacokinetics (PK) to pharmacodynamics (PD) to analyze the variable relationship over time for the discriminative stimulus effects of cocaine and various metabolites which influences conclusions of drug action. This interesting PK/PD relationship allows a unique perspective of potential species differences in the discriminative stimulus effects of drugs.

Taken as a whole, the studies reviewed in this volume are just a fraction of the literature demonstrating the high receptor selectivity, stereoselectivity, and susceptibility to competitive antagonism for drugs trained as discriminative stimuli, the classic receptor pharmacology principles required to define a drug class. In the future, drug discrimination will still be needed to characterize new ligands, new enantiomers, and novel antagonists especially those agents with likely CNS activity. Although radioligand binding assays or functional GPCR assays are clearly the first steps to screen new compounds, a functional assay in a whole animal, such as drug discrimination, will always be needed to validate the results of more molecular characterizations.

2 Drug Discrimination as a Tool to Reveal Complex Cues and Pharmacological Actions

The early characterization of fentanyl as a discriminative stimulus and the corresponding receptor neuropharmacology of this direct acting opioid agonist (Colpaert 2011) led to using training drugs with more indirect or unique mechanisms of action as discriminative stimuli. Indeed, drug discrimination studies were key in distinguishing potential underlying neural mechanisms. For example, drug discrimination studies differentiated the stimulus effects of PCP (phencyclidine) and MK-801 (dizocilpine) as noncompetitive NMDA antagonists as opposed to direct acting NMDA (*N*-methyl-D-aspartate) receptor antagonists revealing a complex or compound cue involving the regulation of dopaminergic and serotoninergic systems with sigma1 receptor function likely involved (Mori and Suzuki 2016). Psychostimulants, such as cocaine, amphetamine, and more recently synthetic cathinones that possess a mix of transporter inhibition, release, or reverse transporters have been trained as discriminative stimulus and reviewed in this volume (Berquist and Fantegrossi 2017). Drug discrimination techniques can be very useful for studying and classifying opioids with complex pharmacology at multiple receptors, as these can vary significantly across species due to likely different receptor proportions or signaling across species (e.g., Zhu et al. 1997). Drug discrimination techniques have been critical for understanding the role of endogenous cannabinoids and their various metabolic activities and for the pharmacological effects of phytocannabinoids and the synthetic cannabinoid agents (Wiley et al. 2016). For example,

the complexity that can be revealed by training metabolic enzyme inhibitors as a discriminative stimuli to tap into endocannabinoid function was recently demonstrated by training SA-57, a dual fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase101 (MAGL) inhibitor and by training selective MAGL inhibitor MJN110. Using the patterns of substitution for other dual FAAH/MAGL, MAGL, or FAAH inhibitors to substitute for MJN110 as well as cannabinoid agonists, these authors suggest that the MJN110 discriminative stimulus through selective MAGL inhibition is mediated through 2-AG-mediated stimulation of CB1 receptors. Furthermore, under normal endogenous conditions, MAGL may reduce endocannabinoid-mediated overstimulation of the CB1 receptor, thereby preventing induction of a cannabimimetic subjective state (Owens et al. 2017). This example and others reviewed in this volume highlight the manner in which investigators can use drug discrimination techniques to enhance our understanding of the roles of endogenous regulators of drug action.

There are numerous examples highlighted in the current volume that reveal certain drugs can have compound pharmacological cues and drug discrimination methodology has been used to dissect the relative contributions of each component. For example, cocaine, scopolamine, and D_1 and D_2 agonists substitute for the bupropion discriminative stimulus, and these effects were either fully or partially blocked by DA receptor antagonists (Prus and Porter 2016). Similarly, the discriminative stimulus effects of competitive and noncompetitive NMDA receptor antagonists tap into dopaminergic and serotonergic systems as well as sigma-1 receptor actions suggesting that training these agents can result in a compound cue (Mori and Suzuki 2016). Inhalants as a class of discriminative stimuli also fall into the category of interacting with multiple receptor systems such as $GABA_A$-positive modulators and NMDA, for example, depending on the particular inhalant trained as the discriminative stimulus (Shelton 2016). Using drug discrimination to characterize the inhalants allows an investigator to meaningfully group these substance inhalants together despite being such a heterogeneous pharmacological group. The most studied complex discriminative stimulus is ethanol in which GABA (*gamma*-aminobutyric acid) and glutamate ionotropic receptors and serotonergic mechanisms all contribute to the discriminative stimulus effects especially dependent on training dose (Allen et al. 2017). Indeed, there have been clever control experiments designed to separate exteroceptive vs. interoceptive cue components such as route of administration studies to eliminate odor as providing a key role in the discriminative stimulus effects of toluene as reviewed by Shelton (2016).

Interestingly, drug discrimination procedures can be modified to further separate out complex cues for drugs with overlapping pharmacological mechanisms by training dose-dose or three-choice discriminations. For example, Berquist and Fantegrossi (2017) nicely review the usefulness of three-choice discriminations, especially for analyzing the effects of enantiomers in the substitution patterns of MDMA (3,4-methylenedioxymethamphetamine, commonly known as ecstasy). Three-choice discriminations for MDMA, saline, and d-amphetamine reveal a likely serotoninergic-dopaminergic continuum for the underlying neuropharmacological mechanisms of MDMA (Harper et al. 2011; Goodwin and Baker 2000) based on

substitution patterns of different psychostimulants and doses. Three-choice discriminations can also be established with high and low doses of drugs to parcel out the role of efficacy in discriminative stimulus effects (e.g., Jones et al. 1999; Vanecek and Young 1995). Leveraging different mouse strains to further triangulate on components of a complex discriminative stimulus such as clozapine has been a fruitful strategy (Porter et al. 2017) essentially similar to varying a training dose. Narrowing the conditions under which generalization will occur with each new cue or dose that can be trained is a sophisticated strategy to dissect out pharmacological mechanisms under particular contingencies and may explain individual subject substitution patterns.

The observation that individuals can attend to one component of a complex cue more than others has precedence in the literature. In a classic experiment, Reynolds (1961) demonstrated that when two individual pigeons were trained to respond in the presence of a white triangle on a red key and tested with either the triangle or red background alone, one pigeon exclusively attended to the triangle while the other the red background. Drugs with multiple pharmacological components could certainly serve similar functions in individual subjects so that in a group of subjects, some could attend more to one component of the complex stimulus or the other or perhaps even only the Gestalt of the multiple components together. Possibly, component pharmacology or cues could be a contributing factor to some of the inter-subject variability obtained in drug discrimination experiments and one of the reasons examining a pattern of substitution and antagonism in individual subjects is an important part of data analysis in this field. Indeed, this notion has been well-studied by researchers investigating mixtures of drugs (e.g., Stolerman et al. 1999).

3 Drug Discrimination to Study Internal States

Whereas the use of drug discrimination to understand contributions of complex underlying pharmacological mechanisms to drug effects has been invaluable to researchers, one may argue that the ability of discrimination methodologies to tap into the various interoceptive effects of drug stimuli that control behavior makes it a unique procedure without parallel. As described in the first chapter of this volume, drug discrimination grew out of the interest in the effects of drugs on memory retrieval and state-dependent learning (Porter et al. 2018). Two examples of "states" produced by drugs, or the withdrawal of drugs, are worth mentioning because these examples reveal what is especially novel about the results from drug discrimination studies. Rosecrans and Young (2017) reviewed a study in which rats were trained to discriminate pentylenetetrazol from saline and suggested that the basis for the discrimination was pentylenetetrazol-induced anxiety (Harris et al. 1986). When the pentylenetetrazol-trained rats were administered high doses of nicotine for a 3-week period and then were withdrawn from nicotine dosing, the rats responded partially on the pentylenetetrazol-appropriate lever 24 h after the cessation of dosing. These investigators suggested that rats in nicotine withdrawal may be experiencing

"anxiety" as measured by their pentylenetetrazol generalization response. The possibility that pentylenetetrazol as a discriminative stimulus may represent a state akin to anxiety in animals was followed up with additional pharmacological characterization (Jung et al. 2002), and ethologically relevant drug discrimination experiments demonstrating an interoceptive state associated with species-specific defense reactions in rats produced by exposure to cat predators were similar to the discriminative stimulus cues produced by pentylenetetrazol (Gauvin and Holloway 1991).

Other withdrawal states have been modelled in drug discrimination, including those from repeated agonist administration followed up by later discrimination training sessions with antagonists. Excellent examples include experiments where opioid withdrawal substitutes for the discriminative stimulus effects of naltrexone (e.g., Becker et al. 2008) or partial agonist nalbuphine (Walker et al. 2004) and THC withdrawal substitutes for the discriminative stimulus effects of cannabinoid antagonist rimonabant (e.g., Stewart and McMahon 2010). Peptides and drugs with potential anorexic effects have been tested in rats trained to discriminate between 22- and 2-h food deprivations, a methodology of studying the internal state of "hunger" (Jewett et al. 2006, 2009).

Antagonists in general can be difficult to train as discriminative stimuli although there is a long history of training and testing antipsychotic agents (Prus and Porter 2016; Porter 2011) and noncompetitive NMDA antagonists (Balster 1991; Koek 1999). Often many of these antagonists reveal complex, compound cues which may or may not be reversed by agonist administration and the cue may depend on the species studied (Porter 2011). For some drug classes, modifications of procedures are employed such as maintaining the subjects dependent on an agonist as described above. The maintenance of a subject on chronic agonist treatment induces a certain change in homeostasis or an increase in endogenous tone that can be disrupted with antagonists or drug withdrawal. Another modification of the drug discrimination assay to train antagonists such as phencyclidine, diprenorphine, naloxone, naltrexone, and rimonabant as discriminative stimuli without chronic agonist treatment is the conditioned taste aversion methodology reviewed by Riley et al. (2016). One possibility for the establishment of antagonists as discriminative stimuli to control behavior has been suggested to be the disruption of an endogenous tone by the antagonist. In drug-naïve subjects, one might simply suspect basal endogenous tone would be the same after the injection of a given dose of antagonist irrespective of the training procedure. Yet, antagonists can easily serve as discriminative stimuli to control taste aversion learning at lower doses than previously attempted using operant-based training techniques, and these antagonist doses can be trained much more quickly using conditioned taste aversion. These studies demonstrate that the discriminative stimulus properties of a drug are not inviolate properties of the pharmacology but more so intimately tied to the training conditions and predictive consequences of that discriminative stimulus.

In humans, investigators are able to compare subjective effect questionnaires to the results obtained from drug discrimination assays allowing for an assessment of whether drug discrimination is a model of subjective effects. Overall, there is a relatively good correspondence between the discriminative stimulus and subjective

effects in humans across the different pharmacological classes; however, there are some interesting exceptions. Bolin et al. (2016) provide an interesting discussion regarding the face validity and some potential limitations of drug discrimination procedures in humans for studying the abuse potential of drugs (see also McMahon 2015). For example, drug discrimination in humans is relatively insensitive to circulating blood levels of drug such that the time course of the discriminative stimulus effects, or the proportion of responses to the drug-appropriate option, does not always follow the measured blood levels (Kelly et al. 1997). Although we believe that humans are able to articulate the stimuli that may be controlling their behavior, this is probably an overstatement. For example, in humans responding to receive i.m. injections of morphine, much lower doses of morphine were self-administered as compared to those doses that occasioned positive reports of subjective drug effects (Lamb et al. 1991). The notion that to be a discriminative stimulus, a drug must produce something akin to a subjective effect leaves out some discriminative stimuli that likely do not possess strong subjective effects. For example, MAO inhibitors such as iproniazid, nialamide, phenelzine, and tranylcypromine can be discriminated using a T-maze procedure (Overton 1982), and Ca++ channel blockers can be discriminative stimuli in traditional operant procedures (Schechter 1995) when these agents are not likely to have what would be considered strong subjective effects. Finally, the observation that antidepressants can be trained in rats and mice that are not depressed suggests that the underlying pharmacology of these agents interacts with underlying basal states to support a salient enough stimulus to control behavior (Prus and Porter 2016) and reveal how the drug discrimination procedure is an exceedingly sensitive methodology.

4 In Praise of Drug Discrimination

As outlined in the many chapters of this volume, there are few experimental models we have available today that are as pharmacologically selective, sensitive, and such an objective measure an interoceptive state in an organism. As Berquist and Fantegrossi (2017) state in the current volume, “Nevertheless, the drug discrimination assay, in its most basic form, reveals pharmacological effects that occur within the central nervous system in species that display little to no verbal communication. We consider this an achievement in scientific research in general, and we submit that the drug discrimination approach is among the most useful in vivo analyses available to behavioral pharmacology.” Drug discrimination is essentially unchallenged as a method to characterize drug stimuli and resulting behavior. Even with the advanced technologies available today, the ability to study pharmacologically and disease-relevant doses with such specificity in a preclinical experiment is readily available using drug discrimination. Drug discrimination will likely continue to contribute to our understanding of drug-receptor interactions and basic pharmacological characterization in combination with other technologies such as imaging, optogenetics, gene delivery strategies, RNA interference technology, and

designer receptors exclusively activated by designer drugs (DREADD)-based chemogenetic tools. All of these more recent technologies provide exquisite detail on molecular and cellular signaling and brain circuitry; however, to deliver a representation of either drug stimuli or internal states of physiology, a particular cue will have to be specifically trained in an experimental animal. For any question that requires a functional output and a precise, selective pharmacological result, drug discrimination will always be the answer. The only limitation is our creativity.

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