Hallucinogens in Drug Discrimination

Lisa E. Baker

Abstract Hallucinogens comprise a diverse collection of chemicals with multifarious receptor actions in the central nervous system. Preclinical drug screening methods have proven invaluable in the evaluation and characterization of hallucinogen psychopharmacology. Used in concert with structural chemistry and receptor pharmacology methods, preclinical drug discrimination research has informed our current understanding of hallucinogens and the neurochemical receptor mechanisms responsible for their interoceptive stimulus effects. This chapter summarizes the strengths and limitations of drug discrimination as an in vivo drug detection method and offers a brief review of historical and contemporary drug discrimination research with classical hallucinogens.

Keywords Hallucinogens · Preclinical drug screening · Drug discrimination methods · Psychopharmacology · Behavioral pharmacology

Contents

L.E. Baker (✉)
Department of Psychology, Western Michigan University, Kalamazoo, MI, USA
e-mail: lisa.baker@wmich.edu

Curr Topics Behav Neurosci (2017) 36: 201–219
DOI 10.1007/7854_2017_476
Published Online: 09 May 2017

1 Introduction and Overview

Hallucinogens, broadly defined, comprise a diverse collection of psychoactive drugs that have been categorized into a variety of subclasses based on chemical structures, pharmacological actions, and subject-reported psychological effects. Considerable overlap exists among these classification domains. As such, hallucinogens with similar pharmacological actions in the central nervous system (CNS) generally tend to produce similar subjective effects, although there are notable exceptions. The application of behavioral screening methods utilizing nonhuman models has proven invaluable in elucidating the links among chemical structures, receptor pharmacology, and the psychological effects of hallucinogens. Among the various preclinical drug screening tools available, the drug discrimination paradigm is a particularly robust, sensitive, and specific model (Appel et al. 1982). The primary strengths of this behavioral assay include pharmacological specificity and predictive validity.

While no single method is solely sufficient to characterize the behavioral or pharmacological mechanisms of drug action, drug discrimination can be a very powerful tool, especially when it is used in concert with other research methods, such as in vitro assays of receptor binding or intracellular signaling. This chapter describes the drug discrimination paradigm with an emphasis on methodological variables, and offers a brief review of historical and contemporary research on the evaluation of classical hallucinogens utilizing these methods. Examples of research in which drug discrimination methods are paired with methods of structural chemistry and pharmacology are emphasized. The chapter ends with suggestions for future avenues of research.

2 Hallucinogen Classifications

The classical hallucinogens consist of two broad categories, indolealkylamines (also called indoleamines) and phenylalkylamines, each with subclassifications based on structural differences (Glennon 1994). The subclasses of indolealkylamines include simple tryptamines [e.g., *N,N*-dimethyltryptamine (DMT)], methyltryptamines (e.g., 5-methoxy-α-methyltryptamine), ergolines [e.g., lysergic acid diethylamide (LSD)], and ß-carbolines (e.g., harmala alkaloids). The phenylalkylamines consist of phenethylamines (e.g., mescaline) and phenylisopropylamines [e.g., 1-(2,5-dimethoxy-4-methyl-phenyl)-2-aminopropane (DOM)]. A more recent review on the behavioral pharmacology of hallucinogens offers a simpler distinction between two main chemical classes of hallucinogens, based on the structural backbone of either phenethylamine or tryptamine (Fantegrossi et al. 2008), both subclasses of the broader categories previously delineated by Glennon (1994). According to this simpler classification, DOM, and other related compounds, DOB and DOI are all characterized as phenethylamines. Other phenethylamines are noted for their structural similarities to the CNS stimulant,

amphetamine [3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyamphetamine (MDA)]. Although these substances are sometimes broadly classified as hallucinogens, they are more appropriately designated as emphathogens or entactogens (Nichols 1986).

Other categories of hallucinogens, structurally and pharmacologically distinct from the classical hallucinogens, include the dissociative anesthetics [e.g., ketamine, phencyclidine (PCP)], anticholinergics (e.g., scopolamine, atropine), and kappa-opioid receptor agonists (e.g., salvinorin A). For the sake of brevity, this chapter emphasizes drug discrimination research with the classical (serotonergic) hallucinogens.

3 The Drug Discrimination Paradigm

Drug discrimination is a well-established experimental method, commonly employed to classify psychoactive drugs and to characterize their neuropharmacological actions. This method has been utilized with nonhuman subjects (rats, mice, nonhuman primates) and human research participants. With some exceptions, there is considerable overlap across species regarding the discriminative stimulus effects of hallucinogens. The drug discrimination paradigm takes advantage of the fact that psychoactive drugs produce physiological changes in the nervous system that can function as interoceptive stimuli. Through differential reinforcement of specific behaviors (e.g., a lever press) in the presence or absence of such stimuli, an organism can be trained to indicate when these stimuli are present. Thus, the drug discrimination paradigm offers a rigorous and robust method for in vivo drug detection.

Of particular importance to the validity of drug discrimination as an in vivo drug-detection method, psychoactive drugs with similar discriminative stimulus effects in nonhumans tend to also produce similar subject-reported psychological effects in humans with considerable reliability (Young 2009). For example, responses in animals trained to discriminate lysergic acid diethylamide (LSD) reliably generalize to other classical hallucinogens (e.g., psilocybin, mescaline, or DMT), but do not typically generalize to the dissociative anesthetics (e.g., phencyclidine or ketamine) or to other psychoactive drug classes, such as central nervous system (CNS) stimulants, sedatives, or opiate analgesics. The entactogens (e.g., MDMA, MDA) have been reported to produce similar discriminative stimulus effects to LSD, although research findings are not always consistent regarding these similarities (Oberlender and Nichols 1988; Schechter 1998; Callahan and Appel 1988; Goodwin et al. 2003).

Indeed, drug discrimination is a particularly attractive investigative paradigm due to the specificity of discriminative stimuli correlating with underlying cellular and molecular mechanisms of drug action (Holtzman and Locke 1988; Colpaert 1999). For example, while mescaline (a phenethylamine derivative) is structurally different from LSD (an ergoline), these drugs share similar pharmacological actions

at 5-HT_{2A} receptors and also reliably substitute for one another in nonhuman drug discrimination experiments (Appel and Callahan 1989). Such findings are consistent with reports that mescaline and LSD produce similar subject-reported psychological effects in humans (Winter 2009). Specific examples of mechanistic studies utilizing drug discrimination methods are addressed later in this chapter.

4 Drug Discrimination Methodology

A variety of behavioral strategies have been employed to establish discriminative stimulus control with psychoactive drugs in nonhumans, some utilizing classical conditioning methods (e.g., conditioned taste aversion) and others using operant conditioning methods with either negative reinforcement (e.g., conditioned shock avoidance), or positive reinforcement (e.g., appetitive conditioning). In contemporary drug discrimination research, the most commonly employed drug discrimination methods utilize operant conditioning technology in which food-restricted animals (rats, mice, or nonhuman primates) are reinforced with food delivery for responding on one operandum (e.g., pressing a lever in an operant conditioning chamber) within a specified time period following drug injections and they are reinforced for emitting an alternate response (pressing a different lever) following vehicle (e.g., saline) injections. Drug or vehicle training sessions typically occur once per day, in an alternating, semi-random order.

The specific training methods and the mastery criteria for stimulus control vary among drug discrimination studies from different laboratories. One methodological variable of interest is the schedule of reinforcement used to establish the discrimination. Variable interval (VI) and fixed ratio (FR) schedules are two common reinforcement schedules employed in drug discrimination with nonhumans, so will be discussed briefly here. The key difference between interval and ratio schedules is what determines the delivery of reinforcement, which in turn influences the frequency of reinforcement. With interval schedules, the delivery of reinforcement is dependent on passage of time; with variable interval schedules, the passage of time is unpredictable. For example, in a VI 30-s schedule, the average time interval between opportunities to earn a reinforcer is 30 s, but it may be shorter or longer. Training for an extended period under a VI reinforcement schedule tends to produce a moderate but steady response rate, with minimal pausing after reinforcement delivery. A key advantage of VI schedules is that they are resistant to extinction. With ratio schedules, the delivery of reinforcement is dependent on the number of responses emitted by the organism. For example, an FR 20 schedule requires the organism to emit 20 responses to receive a reinforcer. This schedule engenders a high, steady response rate until the delivery reinforcement, with a brief response pause following each reinforcer delivery.

It has long been established that reinforcement schedules can influence the development of discriminative stimulus control by drugs (Overton 1979; Koek and Slangen 1982; Stolerman 1989; McMillan and Wenger 1984; McMillan et al. 2001).

While these studies are of historical significance to drug discrimination researchers, investigations on the influence of reinforcement schedule or other methodological variables are extremely scarce in the drug discrimination literature with hallucinogens. Kueh and Baker (2007) compared FR 20 and VI 15-sec reinforcement schedules on the acquisition of discriminative stimulus control by MDMA. While stimulus control was established more rapidly under the FR 20 schedule, only minor differences were observed in MDMA dose-response functions. However, LSD produced a higher percentage of MDMA-lever appropriate responding in rats trained to discriminate MDMA on the VI 15-sec schedule compared to rats trained on the FR 20 schedule. This finding suggests training methods can influence the outcome of stimulus generalization tests.

For good reason, the majority of drug discrimination research with hallucinogens has emphasized pharmacological questions, in an attempt to discern the neural systems and receptor-mediated activities contributing to the interoceptive stimulus effects of these drugs. However, the possibility that training methods can influence the outcome of these studies should not be ignored. Inasmuch as training methods can be conceptualized as a convenient manipulation of behavioral history, inquiry into the behavioral mechanisms involved in hallucinogen discrimination may warrant further investigation.

When comparing the results obtained from different laboratories, it is important to attend to certain methodological differences. In addition to differences in reinforcement schedules as noted above, the criteria required to establish stimulus control also vary among studies. Mastery criteria for stimulus control typically range between 75 and 83% for a specified number of training sessions (e.g., five consecutive sessions or eight out of 10 consecutive sessions). Most studies include the criteria that discrimination accuracy be attained prior to the delivery of the first reinforcer in each session as well as for the remainder of each training session before commencing stimulus generalization tests. Once stimulus control is established with a training drug, stimulus generalization tests are then conducted with a range of doses of that substance as well as several other substances. Some investigators conduct these tests under extinction, not allowing for reinforcement of responding under potentially different stimulus conditions. Other investigators allow for programmed reinforcement of responses on either lever during these assessments.

The results obtained from stimulus generalization tests are typically used to plot dose-response curves for quantitative comparisons. For example, dose-response curves may be compared with respect to the magnitude of stimulus generalization, or height of the dose-response curves, to determine whether a test drug substitutes for the training drug. The magnitude of stimulus generalization can be expressed as the average percentage of drug-lever responses among the animals tested or as the percentage of animals that selected the drug lever. The former is referred to as a quantitative measure, while the latter is referred to as a quantal measure. Both measures provide an index of similarity between the interoceptive stimulus effects of the training drug and the test drugs. Most researchers generally consider a minimum of 80% drug-lever responses or 80% of rats selecting the drug lever as evidence for full stimulus generalization (or substitution).

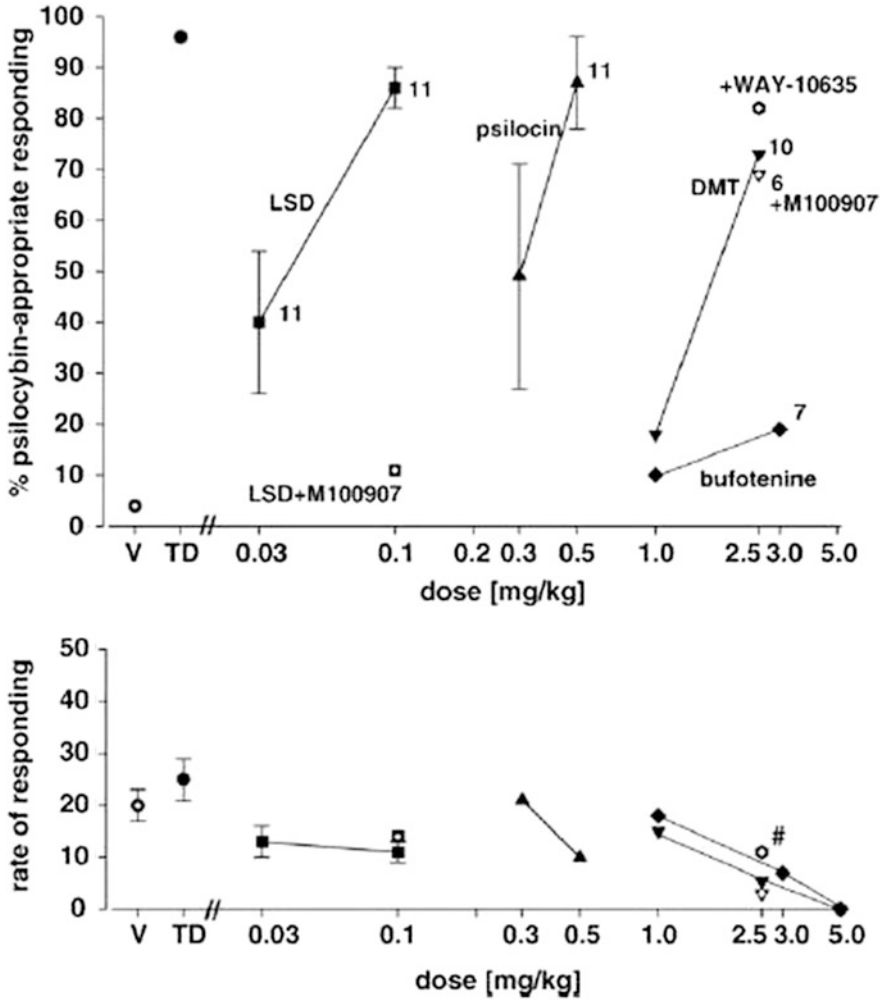

Fig. 1 Reprinted from Winter et al. (2007)

The term "generalization" is often used incorrectly and interchangeably with the term "substitution" in the drug discrimination literature. The following two sentences differentiate the proper use of these terms. Responses may *generalize* from one stimulus to other stimuli. A stimulus may *substitute* for other stimuli. In the case of drug discrimination, responses are the allocation of lever presses and the stimuli are drug-induced interoceptive stimuli. An example of stimulus generalization (i.e., drug substitution) is depicted in Fig. 1, reprinted from a study reported by Winter et al. (2007). This figure illustrates dose-response curves obtained with LSD, psilocin, DMT, and bufotenine in rats trained to discriminate 0.5 mg/kg psilocybin. LSD and psilocin produced full substitution for psilocybin,

whereas DMT produced only 73% psilocybin-appropriate responding and bufotenine produced less than 20% psilocybin-appropriate responding at the doses tested.

Dose-response curves generated from drug substitution tests also allow for comparison of drug potency. The median-effective dose (ED_{50}) may be estimated from a linear regression of dose-response curves that are typically plotted on a logarithmic scale. The ED_{50} values are compared among the test drugs and the training drug. Drugs with a lower ED_{50} value are considered more potent than those with a higher ED_{50} value. In the aforementioned example, LSD is more potent than psilocin.

In addition to the assessment of substitution between various test drugs and a particular training drug, response rate is another quantitative measure of interest. As such, response rate can provide an index of drug-induced suppression of behavior. As a general rule, increasing doses of a test drug are assessed until either full substitution is observed or until response rate is significantly reduced. For example, a study by Killinger et al. (2010) assessed salvinorin A, a unique hallucinogen with selective kappa receptor affinity in animals trained to discriminate LSD. Figure 2 depicts the dose-response function determined for LSD and salvinorin A. LSD produced dose-dependent increases in responding on the LSD-associated lever. Salvinorin A failed to produce more than 40% LSD-lever responding and significantly reduced response rate.

A particularly attractive feature of drug discrimination methodology is that it allows for the assessment of neurochemical actions and/or receptor signaling mechanisms that contribute to the discriminative stimulus effects of a drug. For such assessments, pharmacological antagonists are given in combination with the training drug or in combination with another drug that substituted for the training drug to ascertain whether their stimulus effects can be attenuated or blocked. For example, several different drugs with varying receptor affinities and selectivity can be compared for antagonism of the drug stimulus. In one such study, Fiorella et al. (1995a) evaluated 12 different pharmacological antagonists with varying affinities for 5-HT_{2A} and 5-HT_{2C} receptors. Each antagonist was assessed in combination

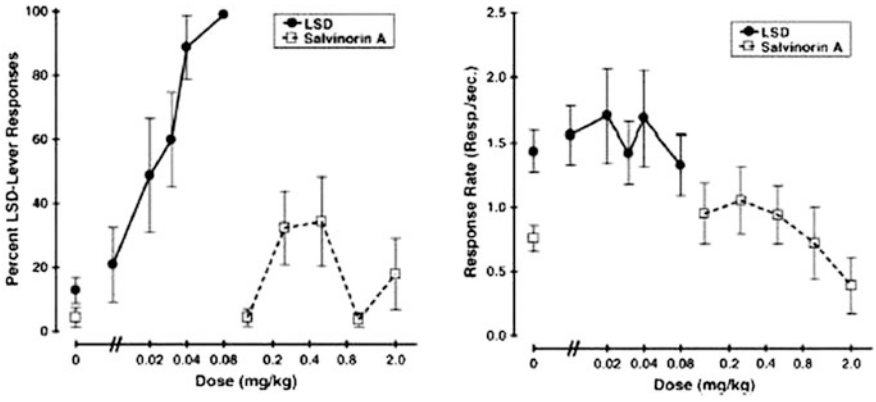

Fig. 2 Reprinted from Killinger et al. (2010)

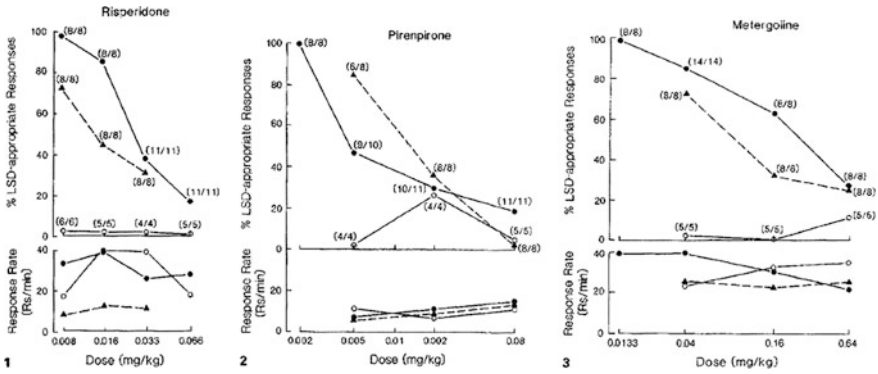

Fig. 3 Reprinted from Fiorella et al. (1995a)

with LSD or (−)-DOM in rats trained to discriminate LSD. For illustration, Fig. 3 depicts three of the 12 dose-response curves reported in that study. These graphs depict the dose-inhibition functions for risperidone, pirenpirone, and metergoline. The open circles represent the effects of each antagonist alone. The closed circles represent each drug in the presence of 0.1 mg/kg LSD, and the closed triangles represent each antagonist in the presence of 0.4 mg/kg (−)-DOM. The results of this study are discussed later in this chapter under the heading Mechanistic Studies.

Another common strategy in drug discrimination studies involves the assessment of pharmacological agents in combination with the training drug to determine whether they potentiate its effects. For example, although the noncompetitive NMDA glutamate antagonists do not fully substitute for serotonergic hallucinogens, these substances have been shown to potentiate the effects of DOM and LSD (Winter et al. 2000, 2004).

Despite the obvious limitations inherent in any attempt to model a complex and uniquely human subjective experience using infrahuman species, the strengths of drug discrimination research with hallucinogens are irrefutable. When paired with tests of pharmacological agents with varying receptor selectivity and affinity, drug discrimination studies have aided in elucidating multiple neurotransmitter receptor subtypes contributing to the complex interoceptive stimuli produced by hallucinogens. Considering the recent resurgence in the recreational use as well renewed interests in the therapeutic potential of some hallucinogens, preclinical drug discrimination studies continue to inform clinical investigations of these drugs.

5 Preclinical Drug Discrimination with Classical Hallucinogens

An exhaustive review of drug discrimination research on hallucinogens over the past four decades is beyond the scope of this chapter. However, some prominent examples of early research with classical hallucinogens (e.g., LSD, mescaline,

psilocybin) and a few leading examples of contemporary research will be highlighted. Hirschhorn and Winter (1971) published the first known report that LSD and mescaline can establish discriminative stimulus control in rats. Subsequent research over the next decade revealed that the discriminative stimulus effects of mescaline and LSD are distinct from hallucinogens outside the indoleamine or phenethylamine subclasses (Shannon 1981; Silverman and Ho 1978; Swedberg and Jarbe 1986). Early studies also established that psilocybin induces stimulus control in rats (Harris and Balster 1971; Schechter and Rosecrans 1972; Koerner and Appel 1982).

Research findings accumulated over the past four decades indicate that drug-induced stimulus control by the classical hallucinogens (i.e., LSD, phenethylamines, tryptamines) is primarily dependent on their interactions with serotonin (5-HT) receptors (Appel et al. 1982; Colpaert et al. 1982; Glennon et al. 1982, 1984a, b; Kuhn et al. 1978; Winter 1978; Young et al. 1982). Despite numerous mechanistic studies on the interoceptive stimulus effects of these hallucinogens, the precise mechanism of action underlying these effects remains elusive. The 5-HT_{2A} receptor subtype is thought to be necessary, but not sufficient, and there is considerable evidence that both the 5-HT_{2C} and 5-HT_{1A} receptor subtypes also contribute to the interoceptive stimulus effects of these substances (Fiorella et al. 1995a; Winter 2009; Carbonaro et al. 2015; also see Nichols 2004 and Fantegrossi et al. 2008 for reviews).

Winter et al. (2007) were the first researchers to examine thoroughly the involvement of serotonin receptors in psilocybin discrimination in the rat. In tests of stimulus generalization, DOM, LSD, psilocin, and DMT all substituted fully for psilocybin, while only partial substitution was observed with 2C-T-7 (2,5-dimethoxy-4-propylthiophenethylamine) and mescaline. These authors also reported that MDL 100907, a 5-HT_{2A} inverse agonist, partially blocked psilocybin discrimination, whereas the 5-$HT_{1A/7}$ receptor antagonist, WAY-100635, and the dopamine D_2 receptor antagonist remoxipride failed to block psilocybin discrimination. Winter et al. (2007) concluded that 5-HT_{2A} receptor activities play a prominent but incomplete role in the compound stimulus induced by psilocybin. They further noted that unlike other closely related hallucinogens, 5-HT_{1A} receptors do not appear to contribute to psilocybin-induced stimulus control.

Despite an abundance of evidence for the involvement of serotonin receptors in the discriminative stimulus effects of the classical hallucinogens, a myriad of research data indicate that interactions among multiple receptor systems contribute to their psychopharmacological effects. For example, González-Maeso et al. (2007) demonstrated that the effects of the tryptamine hallucinogens are dependent on secondary signaling pathways and are not limited simply to 5-HT_{2A} receptor activation. Of particular interest is evidence indicating the involvement of group II glutamate receptors (mGluR2/3) in mediating hallucinogenic effects (González-Maeso et al. 2007, 2008; Delille et al. 2012; Moreno et al. 2011; Winter et al. 2004; Carbonaro et al. 2015).

In a recent study, Carbonaro et al. (2015) explored the involvement of serotonin and group II glutamate receptors in the discrimination of a naturally occurring

tryptamine hallucinogen DMT and a synthetic analog of this substance, *N,N*-diisopropyltryptamine (DIPT). Two separate groups of 16 rats were trained to discriminate either 5 mg/kg DMT or 5 mg/kg DIPT from saline. Tests were conducted with the 5-HT_{2A} inverse agonist, MDL 100907, the 5-HT_{2C} antagonist, SB242084, the mGluR2/3 agonist, LY379268, and the mGluR2/3 antagonist, LY341495. MDL 100907 fully blocked the discriminative stimulus effects of DMT, whereas SB242084 produced minimal attenuation of the DMT cue. Both MDL 100907 and SB 242084 only partially attenuated DIPT discrimination. LY379268 only partially blocked the discriminative stimulus effects of DMT and had minimal effects on DIPT discrimination, whereas LY341495 potentiated the effects of both DMT and DIPT. The authors concluded that 5-HT_{2A} receptors predominantly mediate the discriminative stimulus effects of DMT and DIPT, while both 5-HT_{2C} and mGluR2 receptors may modulate their discriminative stimulus effects to some extent.

In a brief but very informative review of drug discrimination research on the classical hallucinogens, Winter (2009) highlighted two key factors that complicate the characterization of these drugs: (1) the complexity of the serotonin (5-HT) receptor family and (2) the lack of selective antagonists for specific 5-HT receptor subtypes. Winter's review addresses historical and contemporary research on the neurochemical mediation of stimulus control by hallucinogens, indicating a prominent role for 5-HT_{2A} receptor with significant modulation by 5-HT_{2C} and 5-HT_{1A} receptors. Also noted in this review are active investigations on the contribution of dopamine and glutamate to the complex stimulus effects of the classical hallucinogens. At least three crucial points may be gleaned from Winter's commentary on this research: (1) Hallucinogens represent a diverse collection of agents with compound stimulus properties; (2) selective ligands for various receptor subtypes are essential tools for examining the complex stimulus properties of hallucinogens; and (3) preclinical models of drug discrimination, involving mainly rats and some nonhuman primates, have assisted in the characterization of the uniquely complex stimulus properties of hallucinogens.

The challenges Winter (2009) highlighted still exist in behavioral pharmacology research with hallucinogens. Determining the precise mechanistic interactions among serotonergic, glutamatergic, and dopaminergic brain systems involved in drug-induced psychological phenomena is encumbered by the fact that there are multiple receptor subtypes within each of these systems and there are few highly selective agents for these receptors readily available to most researchers. The application of cellular and molecular biology techniques can aid in the evaluation of these mechanistic interactions. As such, evidence from in vitro electrophysiology studies with rodent cortical tissue slices suggests hallucinogens exert their effects through serotonergic–glutamatergic interactions. For example, 5-HT_{2A} receptor stimulation is correlated with an increase in glutamate-mediated synaptic activity in the rat prefrontal cortex (Lambe et al. 2000; Lambe and Aghajanian 2001). Additionally, dopaminergic D_1/D_5 receptors attenuate glutamatergic activity and oppose the effects of both phenethylamines and tryptamines (Lambe and Aghajanian 2007). Béïque et al. (2007) combined cellular and molecular approaches to examine the mechanisms of interaction between 5-HT_{2A} receptors and

glutamatergic synaptic activity in the prefrontal cortex. They located a subpopulation of pyramidal cells that were strongly excited by 5-HT_{2A} receptor activation, and they suggested that 5-HT_{2A} receptors facilitate intrinsic networks within the PFC.

Translating the findings from in vitro electrophysiology studies into mechanistic explanations of drug-induced psychological experiences requires cautious interpretation. While the cellular and molecular actions of hallucinogens in the brain contribute to discriminative stimulus control by these drugs, contextual features of the training environment must also be considered. Just as we cannot ignore the influence of environmental setting on the subjective experience induced by hallucinogens in human users, we must also consider the influence of environmental context when interpreting mechanistic studies of drug discrimination in nonhuman models. Nevertheless, mechanistic approaches in drug discrimination have proven to be an invaluable tool in determining the receptor mechanisms involved in the complex stimulus properties of hallucinogens.

6 Mechanistic Studies

The power of drug discrimination as an in vivo assay of drug–receptor interactions is particularly evident when it is used in concert with other experimental methods, including in vitro receptor binding assays, intracellular signaling assays, and various methods designed to alter the activities or expression of receptors. As such, the majority of published drug discrimination studies involving hallucinogens have emphasized mechanistic approaches to delineate the neural systems involved in their discrimination.

In a series of cleverly designed mechanistic studies, Winter and colleagues conducted fundamental research to differentiate the respective roles of 5-HT_{2A} and 5-HT_{2C} receptors in the discriminative stimulus effects of LSD and (−)-DOM (Fiorella et al. 1995a, b). Two mechanistic approaches utilized in these studies are reviewed by Winter et al. (1999). The first approach utilizes an antagonist correlational analysis. Using this approach, a diverse group of antagonists are tested for blockade of drug-induced stimulus control. In separate in vitro radioligand competition experiments, receptor affinity values are determined with the same collection of antagonists. The IC_{50} values obtained from tests of stimulus antagonism are then analyzed for correlation with binding affinities determined from the receptor binding experiments. In one such study, 12 different antagonists were assessed with a range of doses for antagonism of LSD discrimination and of stimulus generalization to (−)-DOM in rats trained to discriminate LSD (Fiorella et al. 1995a). A few of the graphs from this study were previously displayed (see Fig. 3) to illustrate an example of antagonism tests in drug discrimination. Receptor affinity values for the same set of antagonists were determined in vitro from radioligand competition experiments. Results revealed that the in vivo potency of antagonists to block LSD discrimination and stimulus generalization to (−)-DOM

was highly correlated with the in vitro binding affinity of these antagonists to 5-HT_{2A} receptors and poorly correlated with affinity for 5-HT_{2C} receptors. The authors concluded that the 5-HT_{2A} receptor subtype plays a predominant role in the stimulus effects of LSD and (−)-DOM.

A second mechanistic approach reviewed by Winter et al. (1999) involves pretreatment with serotonin-depleting agents to assess the consequences of such treatment on LSD discrimination. Citing several landmark studies, Winter et al. (1999) reviewed the evidence that pretreatment with the 5-HT-depleting agent 5,7-dihydroxytryptamine (5,7-DHT) potentiates LSD discrimination (White et al. 1980), increases 5-HT_{2C} receptor density, and upregulates 5-HT_{2C}-mediated phosphoinositide (PI) turnover (Conn et al. 1987; Pranzatelli 1990) but does not upregulate 5-HT_{2A} receptors (Conn and Sanders-Bush 1986). Collectively, these findings indicate a modulatory role of 5-HT_{2C} receptors in LSD's stimulus effects. A subsequent study by Fiorella et al. (1995b) tested the hypothesis that 5-HT_{2C} receptor upregulation mediates supersensitivity to LSD discrimination following 5-HT depletion. They compared the effects of two 5-HT-depleting agents, *p*-chlorophenylalanine (PCPA) and *p*-chloroamphetamine (PCA), on LSD discrimination and on 5-HT_{2A} receptor-mediated and 5-HT_{2C} receptor-mediated phosphoinositide (PI) hydrolysis. These chemical agents reduce brain serotonin by different mechanisms; PCPA blocks 5-HT biosynthesis, whereas PCA produces the loss of presynaptic 5-HT neurons through a toxic mechanism. Pretreatment with PCPA, but not PCA, shifted the LSD dose-response curve to the left, indicative of enhanced LSD discrimination. Further, PCPA, but not PCA, significantly increased 5-HT_{2C} receptor-mediated PI hydrolysis. However, 5-HT_{1A} receptor-mediated PI hydrolysis was not affected by either 5-HT-depleting agent. These results suggest the involvement of 5-HT_{2C} receptor-mediated PI hydrolysis in the discriminative stimulus effects of LSD.

In considering the multiple neural systems involved in hallucinogen discrimination, it is important to note that different neuronal systems may differentially contribute to the stimulus effects of a drug at different post-injection intervals. Drug discrimination methods can readily be applied to explore the temporal dynamics of the interoceptive stimulus effects of drugs. For example, Marona-Lewicka and Nichols (2007) utilized drug discrimination methods to explore the distinction between serotonergically mediated and dopaminergically mediated discriminative stimulus effects of LSD. Rats were trained to discriminate LSD from saline following either a 30 or 90-min preinjection interval, and a variety of agonists and antagonists from distinct pharmacological classes were assessed for substitution or antagonism. Other serotonergic hallucinogens, including psilocin and mescaline, substituted only in the rats trained to discriminate LSD with a 30-min preinjection interval. In contrast, several dopamine receptor agonists either fully or partially substituted for the LSD 90-min discriminative stimulus. The authors concluded that dopaminergically mediated effects play a more prominent role in the delayed temporal effects of LSD.

7 False Positives

Despite its pharmacological specificity, drug discrimination is not without limitations. One commonly cited limitation of this paradigm is the occasional occurrence of false positives, when a substance produces substitution for a training drug, but those two drugs do not typically produce similar subject-reported effects in humans. The antimigraine medication, lisuride, is a frequently cited example of a false positive for LSD discrimination. Lisuride is structurally similar to LSD and has a high affinity for 5-HT_{2A} receptors, but lisuride does not produce hallucinations in humans. Nevertheless, lisuride has been reported to produce full substitution in rats trained to discriminate LSD (White and Appel 1982; Holohean et al. 1982), although others have found only partial substitution (Marona-Lewicka et al. 2002). As noted previously, methodological differences should be considered carefully when comparing results from different laboratories. As such, a variety of methodological differences between these studies, such as the type of reinforcer and reinforcement schedule, the presession injection interval, or the use of quantal versus quantitative measures of drug substitution, could contribute to the discrepant findings regarding LSD and lisuride.

The implementation of alternative drug discrimination methods, such as a three-lever drug discrimination (e.g., LSD–vehicle–lisuride) or a drug versus other (e.g., LSD versus multiple other drug stimuli) discrimination, has proven useful in reducing or eliminating the detection of lisuride as a false positive. For example, Callahan and Appel (1990) successfully trained rats to discriminate lisuride from LSD using a three-lever drug discrimination procedure. In another study, one group of rats was trained to discriminate LSD from saline, while another group was trained to discriminate LSD from either saline, cocaine, or pentobarbital (Appel et al. 1999). When lisuride was tested for substitution in both groups, there were fewer instances of false positives in the second group. These studies exemplify how the sensitivity of the drug discrimination assay is dependent on the discrimination training methods.

Lisuride's substitution for LSD's discriminative stimulus effects in nonhumans is likely due to its high affinity for 5-HT_{2A} receptors. However, González-Maeso et al. (2007) differentiated the actions of LSD and lisuride with regard to their functional selectivity via 5-HT_{2A} receptor-mediated intracellular signaling pathways. This study exemplifies how the methods of molecular biology and biochemistry are essential complementary tools to behavioral studies of hallucinogens. Only when multiple methodological approaches are used in concert can we begin to delineate the precise cellular and molecular mechanisms responsible for mediating the complex psychoactive properties of these drugs.

8 Structure–Activity Relationships

When used in the context of drug development and design, drug discrimination serves well for investigating structure–activity relationships (SAR). The specific aim of SAR studies is to assess the influence of chemical structure on

pharmacological activity. When paired with drug design strategies, drug discrimination methods contribute to SAR investigations by providing both qualitative and quantitative information (Glennon and Young 2011). Besides determining whether a test agent produces qualitatively similar discriminative stimulus effects as the training drug, potency comparisons can be made by comparing the ED_{50} values of various test agents to the training stimulus. A recently published book devoted to drug discrimination by Glennon and Young (2011) includes a chapter on the implementation of this drug-detection method in SAR investigations with hallucinogens. In one example, they highlight a study in which rats were trained to discriminate 1.0 mg/kg DOM and subsequently tested with several methoxy-substituted analogs of the basic phenylisopropylamine structure. For a detailed description and graphic depiction of their findings, the interested reader may consult the chapter by Glennon and Young (2011) or the original research report (Glennon and Young 1982). In short, all of the monomethoxy compounds tested failed to substitute for DOM. Of the six positional isomers of dimethoxyamphetamine (DMA) tested, only 2,4-DMA and 2,5-DMA substituted for DOM, and all five trimethoxyamphetamine (TMA) analogs tested produced full substitution for the DOM training stimulus. The authors concluded that the presence of the 4-methyl group of DOM contributes to the stimulus characteristics and the potency of 2,5-DMA as a DOM-like substance.

9 Alternative Training Methods in Drug Discrimination

The most common application of drug discrimination methodology involves training an organism to distinguish a drug from the absence of that drug (Drug vs. Vehicle). Alternative approaches that are currently underutilized include training a discrimination between two drugs (Drug A vs. Drug B), training a discrimination between one drug versus a variety of other drugs (Drug vs. Other), or training a three-lever discrimination among two different drugs and the absence of either drug (Drug A vs. Vehicle vs. Drug B). As mentioned above, some of these methods were employed to differentiate the stimulus effects of LSD and lisuride. In particular, the three-lever drug discrimination procedure has been reported to be a more sensitive tool with which to investigate the stimulus properties of psychoactive drugs (Stolerman 1993). To briefly elaborate on the utility of the three-lever discrimination method, a series of studies are summarized below in which this method was used to characterize the unique discriminative stimulus effects of the entactogen, MDMA.

Traditional two-lever discrimination procedures were employed in the late 1980s and early 1990s to characterize the interoceptive stimulus effects of MDMA. Some of these studies suggested the optical isomers of MDMA may exert differential stimulus effects and that (+)-MDMA may produce more stimulant-like effects compared to (−)-MDMA. For example, Glennon et al. (1988) reported that (+)-MDMA substituted for *d*-amphetamine, whereas (−)–MDMA failed to do so.

However, Oberlender and Nichols (1988) found neither isomer to substitute for *d*-amphetamine. Baker et al. (1995) trained separate groups of rats to discriminate either (+)-MDMA or (−)-MDMA from saline and tested both stimulants (*d*-amphetamine, cocaine) and hallucinogens (DOM, LSD, mescaline) for substitution. None of the stimulants nor the hallucinogens substituted for (+)-MDMA, and only LSD produced full substitution for (–)–MDMA in that study.

In an effort to further differentiate the MDMA isomers, Baker and Taylor (1997) trained rats to discriminate LSD and *d*-amphetamine from saline using three-lever discrimination methods. Both MDMA isomers produced partial substitution for LSD and failed to substitute for d-amphetamine, indicating the isomers do not produce distinct discriminative stimulus effects as previously suggested. In a subsequent three-lever discrimination study, rats were trained to discriminate MDMA from *d*-amphetamine (Goodwin and Baker 2000). In this study, LSD produced dose-dependent increases in MDMA-lever responses but not quite full substitution (78%) for MDMA. However, full substitution for MDMA was obtained with the 5-HT releaser, fenfluramine, indicating that the serotonergic actions of MDMA were particularly salient in maintaining stimulus control in animals trained to discriminate MDMA from *d*-amphetamine, a dopamine releaser. In a follow-up study, it was determined that rats can also be trained to discriminate MDMA from LSD using a similar three-lever discrimination procedure (Goodwin et al. 2003). In that study, *d*-amphetamine produced only partial substitution for MDMA and fenfluramine still produced complete substitution for MDMA. MDL 100907 only partially blocked the stimulus effects of MDMA, but completely antagonized LSD discrimination in these rats. The dopamine D_2 antagonist haloperidol also failed to block MDMA discrimination. These results indicate that 5-HT release remains a salient feature in MDMA's discriminative stimulus effects, even when rats are trained to discriminate MDMA from another serotonin agonist, LSD. Furthermore, this series of three-lever discrimination studies provided conclusive evidence that MDMA produces complex stimulus effects, distinct from both stimulants and hallucinogens.

10 Future Directions

If this chapter has served its purpose, the reader should be convinced that the drug discrimination paradigm is a valuable investigative tool for assessing the psychopharmacology of hallucinogens. Since its inception, this sensitive and pharmacologically specific in vivo drug-detection method has garnered considerable evidence for the involvement of complex neural systems in the interoceptive stimulus effects of hallucinogens. As technology advances within the fields of structural chemistry, molecular biology, and genetic engineering, pairing these technologies with drug discrimination methods can prove fruitful in the continued quest to understand the complexities of hallucinogens and related psychedelic drugs. For example, recent developments in transgenic and genetic knockout rodent

models can make use of drug discrimination methods to discern the importance of specific gene expression to the interoceptive stimulus effects of selected drugs.

As scientific research progresses with emphasis on rediscovering the medicinal values of hallucinogens, we must take into account individual differences in sensitivity to the putative therapeutic effects as well as possible adverse effects. As such, exploration of genetic and sex differences is a worthwhile endeavor in both preclinical and clinical investigations. To date, nonhuman drug discrimination investigations with hallucinogens have used male subjects exclusively. The evaluation of sex differences in the interoceptive stimulus effects of hallucinogens is a potentially lucrative future research direction. Moreover, greater inclusion of female subjects in preclinical behavioral pharmacology is long overdue. Research employing drug discrimination methods to evaluate genetic and sex differences in concert with structure–activity and mechanistic studies of hallucinogens can serve to inform further clinical investigations with hallucinogens.

References

Appel JB, Callahan PM (1989) Involvement of 5-HT receptor subtypes in the discriminative stimulus properties of mescaline. Eur J Pharmacol 159:41–46

Appel JB, White FJ, Holohean AM (1982) Analyzing mechanism(s) of hallucinogenic drug action with drug discrimination procedures. Neurosci Biobehav Rev 6:529–536

Appel JB, West WB, Rolandi WG, Alici T, Pechersky K (1999) Increasing the selectivity of drug discrimination procedures. Pharmacol Biochem Behav 64:353–358

Baker LE, Taylor MM (1997) Assessment of the MDA and MDMA optical isomers in a stimulant-hallucinogen discrimination. Pharmacol Biochem Behav 57:737–748

Baker LE, Broadbent J, Michael EK, Matthews CA, Metosh RB, West WB, Appel JB (1995) Assessment of the discriminative stimulus effects of the optical isomers of ecstasy (3,4-methylenedioxymethamphetamine; MDMA). Behav Pharmacol 6:263–275

Béïque JC, Imad M, Mladenovic L, Gingrich JA, Andrade R (2007) Mechanism of the 5-hydroxytryptamine 2A receptor-mediated facilitation of synaptic activity in prefrontal cortex. Proc Natl Acad Sci 104(23):9870–9875

Callahan PM, Appel JB (1988) Differences in the stimulus properties of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethamphetamine in animals trained to discriminate hallucinogens from saline. J Pharmacol Exp Ther 246:866–870

Callahan PM, Appel JB (1990) Differentiation between the stimulus effects of LSD and lisuride using a three-choice drug discrimination procedure. Psychopharmacology 100:13–18

Carbonaro TM, Eshleman AJ, Forster MJ, Cheng K, Rice KC, Gatch MB (2015) The role of 5-HT_{2A}, 5-HT_{2C} and mGlu2 receptors in the behavioral effects of tryptamine hallucinogens N, N-dimethyltryptamine and N,N-diisopropyltryptamine in rats and mice. Psychopharmacology 232: 275–284

Colpaert FC (1999) Drug discrimination in neurobiology. Pharmacol Biochem Behav 64:337–345

Colpaert FC, Niemegeers CJ, Janssen PA (1982) A drug discrimination analysis of lysergic acid diethylamide (LSD): in vivo agonist and antagonist effects of purported 5-hydroxytryptamine antagonists and of pirenperone, a LSD-antagonist. J Pharmacol Exp Ther 221:206–214

Conn PJ, Sanders-Bush E (1986) Regulation of serotonin-stimulated phosphoinositide hydrolysis: relation to the 5-HT_2 binding site. J Neurosci 6:3669–3675

Conn PJ, Janowsky A, Sanders-Bush E (1987) Denervation supersensitivity of the 5-HT_{1C} receptors in rat choroid plexus. Brain Res 400:396–398

Delille HK, Becker JM, Burkhardt S, Bleher B, Terstappen GC, Schmidt M, Meyer AH, Unger L, Marek GJ, Mezler M (2012) Heterocomplex formation of 5-HT_{2A}-mGlu2 and its relevance for cellular signaling cascades. Neuropharmacology 62:2184–2191

Fantegrossi WE, Murnane KS, Reissig CJ (2008) The behavioral pharmacology of hallucinogens. Biochem Pharmacol 75:17–33

Fiorella D, Rabin RA, Winter JC (1995a) The role of the 5-H_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs I: antagonist correlation analysis. Psychopharmacology 121:347–356

Fiorella D, Helsley SE, Lorraine DS, Palumbo PA, Rabin RA, Winter JC (1995b) The role of the 5-H_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs III: The mechanistic basis for supersensitivity to the LSD stimulus following serotonin depletion. Psychopharmacology 121:364–372

Glennon RA (1994) Classical hallucinogens: an introductory overview. NIDA Res Monogr 146:4–32

Glennon RA, Young R (1982) Comparison of behavioral properties of di- and tri-methoxyphenylisopropylamines. Pharmacol Biochem Behav 17(4):603–607

Glennon RA, Young R (2011) Drug discrimination and in vivo structure-activity relationships. In: Glennon R, Young R (eds) Drug discrimination: applications to medicinal chemistry and drug studies, 1st edn. Wiley, Hoboken, pp 163–181

Glennon RA, Rosecrans JA, Young R (1982) The use of the drug discrimination paradigm for studying hallucinogenic agents. In: Colpaert FC, Slangen JL (eds) Drug discrimination: applications in CNS pharmacology. Elsevier Biomedical Press, Amsterdam, pp 69–96

Glennon RA, Titeler M, Mckenney JD (1984a) Evidence for 5-HT2 involvement in the mechanism of action of hallucinogenic agents. Life Sci 35:2505–2511

Glennon RA, Rosecrans JA, Young R (1984b) Drug-induced discrimination: a description of the paradigm and a review of its specific application to the study of hallucinogenic agents. Med Res Rev 3:289–376

Glennon RA, Yousif M, Patrick G (1988) Stimulus properties of 1-(3,4-methylenedioxyphenyl)-2-aminopropane (MDA) analogs. Pharmacol Biochem Behav 29:443–449

González-Maeso J, Weisstaub NV, Zhou M, Chan P, Ivic L, Ang R et al (2007) Hallucinogens recruit specific cortical 5-HT_{2A} receptor-mediated signaling pathways to affect behavior. Neuron 53:439–452

González-Maeso J, Ang RL, Yuen T, Chan P, Weisstaub NV, Lopez-Gimenez JF, Zhou M, Okawa Y, Callado LF, Milligan G, Gingrich JA, Filizola M, Meana JJ, Sealfon SC (2008) Identification of a serotonin/glutamate receptor complex implicated in psychosis. Nature 452:93–97

Goodwin AK, Baker LE (2000) A three-choice drug discrimination procedure dissociates the discriminative stimulus effects of MDMA and d-amphetamine. Exp Clin Psychopharmacol 8(3):415–423

Goodwin AK, Pynnonen DM, Baker LE (2003) Serotonergic-dopaminergic mediation of MDMA's discriminative stimulus effects in a three-choice discrimination. Pharmacol Biochem Behav 74:987–995

Harris RT, Balster RL (1971) An analysis of the function of drugs in the stimulus control of operant behavior. In: Thompson T, Pickens R (eds) Stimulus properties of drugs. Appleton-Century Crofts, New York, pp 111–132

Hirschhorn I, Winter JC (1971) Mescaline and LSD as discriminative stimuli. Psychopharmacologia 22:64–71

Holohean AM, White FJ, Appel JB (1982) Dopaminergic and serotonergic mediation of the discriminable effects of ergot alkaloids. Eur J Pharmacol 81(4):595–602

Holtzman SG, Locke KW (1988) Neural mechanisms of drug stimuli: experimental approaches. Psychopharmacology 4:138–153

Killinger BA, Peet MM, Baker LE (2010) Salvinorin A fails to substitute for the discriminative stimulus effects of LSD or ketamine in Sprague-Dawley rats. Pharmacol Biochem Behav 96(3):260–265

Koek W, Slangen JL (1982) Effects of reinforcement differences between drug and saline sessions on discriminative stimulus properties of fentanyl. In: Colpaert FC, Slangen (eds) Drug discrimination: applications in CNS pharmacology. Elsevier, Amsterdam, pp 343–354

Koerner J, Appel JB (1982) Psilocybin as a discriminative stimulus: lack of specificity in an animal behavior model for 'hallucinogens'. Psychopharmacology 76(2):130–135

Kueh D, Baker LE (2007) Reinforcement schedule effects in rats trained to discriminate 3,4-methylenedioxymethamphetamine (MDMA) or cocaine. Psychopharmacology 189:447–457

Kuhn DM, White FJ, Appel JB (1978) the discriminative stimulus properties of LSD: mechanism of action. Neuropharmacology 17:257–263

Lambe EK, Aghajanian GK (2007) Prefrontal cortical network activity: opposite effects of psychedelic hallucinogens and D1/D5 dopamine receptor activation. Neuroscience 145:900–910

Lambe EK, Goldman-Rakic PS, Aghajanian GK (2000) Serotonin induces EPSCs preferentially in layer V pyramidal neurons of the frontal cortex in the rat. Cereb Cortex 10:974–980

Lambe EK, Aghajanian GK (2001) The role of Kv1.2-containing potassium channels in serotonin-induced glutamate release from thalamocortical terminals in rat frontal cortex. J Neurosci 21:9955–9963

Marona-Lewicka D, Nichols DE (2007) Further evidence that the delayed temporal dopaminergic effects of LSD are mediated by a mechanism different than the first temporal phase of action. Pharmacol Biochem Behav 87:453–461

Marona-Lewicka D, Kurrasch-Orbaugh DM, Selken JR, Cumbay MG, Lisnicchia JG, Nichols DE (2002) Re-evaluation of lisuride pharmacology: 5-hydroxytryptamine$_{1A}$ receptor-mediated behavioral effects overlap its other properties in rats. Psychopharmacology 64(1):93–107

McMillan DE, Wenger GR (1984) Bias of phencyclidine discrimination by the schedule of reinforcement. J Exp Anal Behav 42:51–66

McMillan DE, Hardwick WC, Li M (2001) Discrimination of pentobarbital doses and drug mixtures under fixed-ratio and fixed-interval reinforcement schedules. Behav Pharmacol 12(3): 195–208

Moreno JL, Holloway T, Albizu L, Sealfon SC, González-Maeso J (2011) Metabotropic glutamate mGlu2 receptor is necessary for the pharmacological and behavioral effects induced by hallucinogenic 5-HT$_{2A}$ receptor agonists. Neurosci Lett 493:76–79

Nichols DE (1986) Differences between the mechanism of Action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class. Entactogens J Psychoactive Drugs 18(4):305–313

Nichols DE (2004) Hallucinogens. Pharmacol Ther 101:131–181

Oberlender R, Nichols DE (1988) Drug discrimination studies with MDMA and amphetamine. Psychopharmacology 95:71–76

Overton DA (1979) Influence of shaping procedure and schedules on performance in the two-bar drug discrimination task: a methodological report. Psychopharmacology 65:291–298

Pranzatelli MR (1990) Neonatal, 5,7-DHT lesions up-regulate (3H)mesulergine-labelled spinal 5-HT1C binding sites in the rat. Brain Res Bull 25:151–153

Schechter MD (1998) MDMA-like stimulus effects of hallucinogens in male fawn-hooded rats. Pharmacol Biochem Behav 59:265–270

Schechter MD, Rosecrans JA (1972) Lysergic acid diethylamide (LSD) as a discriminative cue: drugs with similar stimulus properties. Psychopharmacologia 26:313–316

Shannon HE (1981) Evaluation of phencyclidine analogs on the basis of their discriminative stimulus properties in the rat. J Pharmacol Exp Ther 216:543–551

Silverman PB & Ho BT (1978) Stimulus properties of DOM: commonality with other hallucinogens. In: Colpaert FC, Rosecrans JA (eds) Stimulus properties of drugs: ten years of progress. Elsevier/North Holland, Amsterdam, pp 189–198

Stolerman IP (1989) Discriminative stimulus effects of nicotine in rats trained under different schedules of reinforcement. Psychopharmacology 97:131–138

Stolerman IP (1993) Drug discrimination. In: Van Haaren F (ed) Methods in behavioral pharmacology. Elsevier Science, Amsterdam, pp 217–224

Swedberg MDB, Jarbe TUC (1986) Drug discrimination procedures: differential characteristics of the drug A vs drug B and the drug A vs drug B vs no drug cases. Psychopharmacology 90:341–346

White FJ, Appel JB (1982) Lysergic acid diethylamide (LSD) and lisuride: Differentiation of their neuropharmacological actions. Science 216:535–537

White FJ, Simmons MA, West KB, Holohean AM, Appel JB (1980) The effect of serotonin depletion on the discriminability of LSD. Pharmacol Biochem Behav 13:567–574

Winter JC (1978) Stimulus properties of phenethylamine hallucinogens and lysergic acid diethylamide: the role of 5-hydroxytryptamine. J Pharmacol Exp Ther 204:416–423

Winter JC, Fiorella DJ, Timineri DM, Filipink RA, Helsley SE, Rabin RA (1999) Serotonergic receptor subtypes and hallucinogen-induced stimulus control. Pharmacol Biochem Behav 64:283–293

Winter JC, Doat M, Rabin RA (2000) Potentiation of DOM-induced stimulus control by non-competitive NMDA antagonists. A link between glutamatergic and serotonergic hypotheses of schizophrenia. Life Sci 68:337–344

Winter JC, Eckler JR, Rabin RA (2004) Serotonergic/glutamatergic interactions: the effects of $mGlu_{2/3}$ receptor ligands in rats trained with LSD and PCP as discriminative stimuli. Psychopharmacology 172:233–240

Winter JC, Rice KC, Amorosi DH, Rabin RA (2007) Psilocybin-induced stimulus control in the rat. Pharmacol Biochem Behav 87:472–480

Winter JC (2009) Hallucinogens as discriminative stimuli in animals: LSD, phenethylamines, and tryptamines. Psychopharmacology 203:251–263

Young R (2009) Drug discrimination. In: Buccafusco JJ (ed) Methods of behavior analysis in neuroscience, 2nd edn. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK5225/

Young R, Rosecrans JA, Glennon RA (1982) Comparative discriminative stimulus effects of 5-methoxy-N, N-dimethoxytryptamine and LSD. Life Sci 30:2057–2062