97

RECENT ADVANCES IN ANIMAL MODELS OF DRUG ADDICTION

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DEFINITIONS AND VALIDATION OF ANIMAL MODELS

Definitions of Drug Addiction

Drug addiction is defined as a compulsion to take a drug with loss of control in limiting intake (33). It is considered a chronic disorder because the risk of relapse remains high even after completion of treatment and prolonged abstinence. In 1968, the term drug dependence replaced that of addiction in the nomenclature of the World Health Organization and the American Psychiatric Association. Defined as a cluster of cognitive, behavioral, and physiologic symptoms indicative of an individual continuing substance use despite significant substance related problems, this term has become the accepted diagnostic term for compulsive use of a psychoactive substance. When defined as described, it is analogous to the term addiction. However, this term should not be confused with physical or psychic dependence, conditions in which the cessation or reduction of drug usage results in a withdrawal syndrome. Withdrawal and tolerance often are associated with compulsive drug use; however, they are not required for drug addiction. Although individuals suffering from chronic pain may develop tolerance to the analgesic effects of an opiate and experience withdrawal symptoms, they do not exhibit signs of compulsive drug-seeking behavior.

The concept of reinforcement has provided the cornerstone for current theories and animal models of drug addiction. A reinforcer is defined operationally as ''any event that increases the probability of a response'' and often is used interchangeably with ''reward.'' In general, drugs function as positive or conditioned reinforcers by virtue of their rewarding effects, and reward often connotes additional attributes of a drug (e.g., pleasure) that cannot be easily defined operationally.

This chapter reviews animal models currently used to examine the neurobiological basis of drug addiction and the role of reinforcement processes in its initiation, maintenance, and reinstatement. Emphasis is place on more recently developed models, and where possible, the models are evaluated in terms of reliability and predictability to the human condition. Potential pitfalls to consider when interpreting data also are discussed.

ANIMAL MODELS OF THE POSITIVE REINFORCING EFFECTS OF DRUGS

Drugs of abuse function as positive reinforcing stimuli; this action has provided the framework for currently used animal models of addiction. It is also clear that humans and experimental animals will readily self-administer these agents in the absence of a withdrawal state. Earlier models of drug reinforcement used operant paradigms in nonhuman primates; however, many of these same paradigms now are utilized in rodents. The use of these rodent models, together with the development of modern neurobiological techniques, has provided important information regarding the neurobiology of addiction (11,15,36,45,51).

Operant Intravenous Drug Self-Administration

Drugs of abuse are readily self-administered intravenously by experimental animals, and, in general, drugs that are self-administered correspond to those that have high abuse potential. However, that not all drugs abused by humans are self-administered by experimental animals (e.g., hallucinogens). Furthermore, there are species- and strain-related differences in the degree to which a drug is self-administered (48,62). A detailed review of intravenous self-administration was presented in the previous edition (46); therefore, only key points are presented here.

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For intravenous self-administration studies, the subject acquires a drug infusion by performing a discrete response. The number and pattern of responding required for each infusion is determined by the schedule of reinforcement imposed by the experimenter. Drug availability typically is signaled by an environmental stimulus. The dependent variables are the number of infusions obtained or the rate of responding during a session. In addition to the intravenous route of administration, the intragastric or oral route can be employed (see the following).

Simple Schedules

In fixed-ratio schedules, the number of responses required for drug infusion is set at a fixed number. In rodents, these schedules generally will not maintain stable responding below a certain unit dose and, within the range of doses that maintains stable responding, self-administration rate is inversely related to dose. Within the range of doses that maintains stable responding, animals increase their self-administration rate as the unit dose is decreased. Conversely, animals reduce their rate of self-administration as the unit dose is increased. In a fixed-interval schedule, the frequency of injections is determined primarily by the interval schedule imposed and not the response rate. Therefore, the use of these two schedules can provide information regarding both the motivational effects of a drug and potential nonspecific motor effects that can confound data interpretation.

Progressive-Ratio Schedules

Progressive-ratio schedules are used to evaluate the reinforcing efficacy of a self-administered drug. In this procedure the response requirements for each successive drug reinforcement are increased and the *breaking point* (the point at which the animal will no longer respond) is determined (89). Breaking points are defined either as the largest ratio requirement that the subject completes or as the number of ratios completed by the subject per session. This value represents the maximum work a subject will perform to receive an infusion of a drug. Because the dependent measure in progressive-ratio schedules is not directly related to the rate of responding, interpretive problems associated with using rate of responding as a measure of reinforcement efficacy are avoided.

This schedule has been used to study the relative reinforcing efficacy of compounds both within and across drug classes (61) as well as the neural basis of drug reinforcement (58,61,78,89). Drug craving has been conceptualized as the incentive motivation to self-administer a drug that has been previously consumed; therefore, this schedule can also provide an animal model of drug craving. However, the breaking point reflects both the unconditioned (reinforcing) and conditioned incentive effects of drugs and does not allow for assessment of drug seeking in the absence of drug administration.

Multiple Schedules

Clinical definitions of drug addiction and dependence typically refer to the disruptive effects of addiction on non—drug-related activities. The use of multiple schedules of reinforcement enables the application of concepts of behavioral economics (e.g., commodities, consumption, price, and demand) to operant behavior. It also can provide a control for nonselective effects of drug reinforcement. In these procedures, self-administration of a drug is incorporated into a multiple component schedule with other reinforcers. Studies using these procedures have shown that the contingencies for concurrent reinforcers can affect behavior asymmetrically and that the response requirement for reinforcers can affect drug self-administration (12). Drugs also can function as substitutes for, complements of, or be independent from, the "price" of one another and can be interpreted in economic terms. In addition to evaluating the selectivity of manipulations that apparently reduce the reinforcing efficacy of a drug, these procedures can provide information regarding those factors affecting "loss of control," as well as behavioral and/or pharmacologic therapies for the treatment of addiction. Behavior maintained by alternate presentation of natural reinforcers (e.g., food and water) and drugs of abuse in the same session and with the same reinforcement requirements has been reported for several species (16,20,97,98).

Second-Order Schedules

In a second-order schedule, completion of an individual component (or part) of the schedule produces the terminal event (drug infusion) according to another overall schedule. Typically, completion of a unit schedule results in the presentation of a brief stimulus, and completion of the overall schedule results in the delivery of the stimulus and the drug. Second-order schedules have the advantage that they maintain high rates of responding and extended sequences of behavior before any drug infusion occurs. Therefore, acute drug effects on response rates are minimized. High response rates are maintained even for doses that decrease rates when several injections are self-administered during a session (43). In addition, this schedule requires extended sequences of behavior, thus, modeling the human condition in which drug taking is preceded by a series of behaviors (e.g., procurement, preparation). Although second-order schedules are more typically used in nonhuman primate studies of addiction, their use in rodents is increasing (8,55,73).

Oral Drug-Self Administration

Oral self-administration has focused largely on alcohol because of the obvious face validity of oral alcohol self-admin-

istration and because intravenous self-administration of alcohol is difficult to sustain in rodents (39).

Home Cage Drinking and Preference

A simple approach to studying the motivation to consume a drug is to measure the volume consumed when a drinking bottle is available in the home cage. These procedures have been particularly useful for characterizing genetic differences in drug preference, most often alcohol preference (53), and for initial studies on the effects of pharmacologic treatments on drug intake and preference. Usually a choice is offered between a drug solution and alternative solutions, one of which is often water, and the proportion of drug intake relative to total intake is calculated as a preference ratio. For two-bottle choice testing of alcohol in mice or rats, animals are singly housed and a bottle containing 10% alcohol and one containing water are placed on each cage. Most commonly, animals are allowed free choice of these drinking solutions for successive 24-hour periods with simultaneous free access to food. However, limited access to the drug can induce high drug intakes in short periods of time. Although alcohol is most often studied with these procedures, similar studies have been done with cocaine (40).

Operant Conditioning

A more ''motivational'' approach is to have animals work to obtain drugs for oral consumption using operant procedures. The advantages of such an approach are numerous. The effort to obtain the substance can be separated from the consummatory response (e.g., drinking) and intake easily can be charted over time. In addition, different schedules of reinforcement can be used to change baseline parameters.

For operant self-administration of alcohol, rats can be trained to lever press for alcohol using a variety of techniques all designed to overcome the aversive taste and after effects of initial exposure to alcohol. One approach involves using a sweetened solution fading procedure (80). Alcohol concentrations are increased to a final concentration of 10% over 20 days, with each concentration being mixed first with saccharin or sucrose and then presented alone. Using this approach, animals can be trained to lever press for concentrations of alcohol up to 40% (80). They will perform on fixed-ratio schedules and progressive-ratio schedules and obtain significant blood alcohol levels in a 30-minute session.

Operant self-administration of oral alcohol has also been validated as a measure of the reinforcing effects of alcohol in primates (93). Similar studies have been published with other drugs of abuse (60,90,93).

Reliability and Predictability of Self-Administration Procedures

Drug self-administration has both reliability and predictive validity. The dependent variable provides a reliable measure of the motivation to obtain drugs (e.g., the amount of work an animal will perform to obtain drug) or, in an alternative framework, in demonstrating that drugs function as powerful reinforcers. Responding maintained by drugs as reinforcers is stable across sessions and can be altered predictably by neurotransmitter antagonists. Intravenous drug self-administration also has predictive validity. Drugs having high reinforcement potential in experimental animals have reinforcing effects in humans as measured by both operant and subjective reports (49).

Potential Pitfalls

As discussed previously, self-administration of a drug can vary as a function of the dose available, species or strain tested and the duration of self-administration sessions. It also is influenced by the availability of alternate reinforcers, the presence or absence of environmental stimuli that signal drug infusions, post-reinforcement interval, and prior history of the subject (54). In the progressive-ratio paradigm, breaking point is influenced by the size of the increment by which each ratio increases as well as the initial response requirement that starts a session. Because self-administration typically results in an inverted U-shaped curve, both leftward and rightward shifts in the dose–effect function will decrease self-administration of some unit doses but simultaneously increase self-administration of other doses. The interpretation of downward shifts in the dose–effect function also is sometimes problematic. Therefore, construction of full dose–effect functions is essential in self-administration studies. Since a given pretreatment may decrease self-administration by having nonspecific effects on behavior (e.g., sedation), the influence of a pretreatment on non-drug reinforcers should be assessed.

Conditioned Place Preference

Conditioned place preference is a classical conditioning procedure in which administration of a drug is paired with one distinct environment and administration of placebo with another. After several environmental pairings, allowing noninjected animals access to both environments and measuring the time spent in each assesses the time spent in each environment. The animal's choice to spend more time in either environment provides a direct measure of the conditioned reinforcing effect of a drug. Animals exhibit a conditioned preference for an environment associated with drugs that function as positive reinforcers (e.g., spend more time in the drug-paired compared to placebo-paired environment) and avoid those that induce aversive states (e.g., conditioned

place aversion). This procedure permits assessment of the conditioning of drug reinforcement and can provide indirect information regarding the positive and negative reinforcing effects of drugs. Place conditioning has been used in conjunction with gene transfer and homologous recombination techniques to delineate the neural basis of drug-induced reinforcement (15,74).

The apparatus used in conditioning experiments consists of two environments that are differentiated from each other on the basis of color, texture, and/or lighting. The distinctiveness of the environments is essential for the development of conditioning. In the unbiased design, the environments are manipulated so that animals differentiate one from the other but do not exhibit an innate preference for either of the place cues. Pairing of drug with a particular environment is counterbalanced and change in the time spent in the drug-paired environment can be directly attributed to the conditioned reinforcing effects of a drug. Although quality control experiments confirming the unbiased nature of the procedure are conducted periodically, experiments do not require a preconditioning phase to assess pretest preferences, thus preventing the potential confound of latent inhibition and decreasing the time necessary for a particular experiment.

In the biased design, animals exhibit a preference for one of the place cues prior to conditioning. A preconditioning phase, in which animals are allowed access to both environments, is necessary to determine the innate preference of each animal. The drug then is paired with the preferred or nonpreferred environment depending on whether the drug is assumed to produce aversive or positive reinforcing effects, respectively. Although this design is used often, data interpretation can be problematic because place preferences may indicate incentive motivational effects of a drug or a decrease in the aversive properties of the least-preferred environment.

Reliability and Predictability of Conditioned Place Preference Procedures

The conditioned place preference paradigm has reliability and validity. Drugs that produce conditioned preferences for the drug-associated environment are those that function as positive reinforcers in other paradigms. Conditioned aversions also are observed in response to drugs that are negative reinforcers or produce aversive or dysphoric states in human subjects (34,66).

Potential Pitfalls

In place conditioning studies, the drug is administered noncontingently and there is evidence that the behavioral and neurochemical effects of abused drugs differ depending on whether drug administration is controlled by the subject.

Route of drug administration, number of environmental pairings, and duration of conditioning sessions (18,23) can profoundly affect place conditioning and should be controlled for. Because tests of conditioning are conducted in the absence of drug, the issue of state-dependency also must be addressed.

Place conditioning now is used in many studies assessing genotype-dependent differences in drug sensitivity. However, a lack of a conditioned response may indicate a loss of the reinforcing effects of a drug or a generalized impairment of learning or memory processes required for the acquisition or performance of a conditioned response. In addition, genotype-dependent differences in the saliency of environmental cues used for conditioning may occur. Finally, issues of interpretation and latent inhibition limit the utility of biased place conditioning procedures.

Brain Stimulation Reward Thresholds

Electrical self-stimulation of certain brain areas is rewarding for animals and humans as demonstrated by the fact that subjects will readily self-administer the stimulation (69). The powerful nature of the reward effect produced by intracranial self-stimulation (ICSS) is indicated by the behavioral characteristics of the ICSS response, which include rapid learning and vigorous execution of the stimulation-producing behavior. (See ref. 28 for review.) The high reward value of ICSS has led to the hypothesis that ICSS directly activates neuronal circuits that are activated by conventional reinforcers (e.g., food, water, and sex). In bypassing much of the input side of these neuronal circuit(s), ICSS provides a unique tool to investigate the influence of various substances on reward and reinforcement processes. ICSS differs significantly from drug self-administration in that, in the ICSS procedure, the animal is working to directly stimulate presumed reinforcement circuits in the brain, and the effects of the drugs are assessed on these reward thresholds. Drugs of abuse decrease thresholds for ICSS, and there is a good correspondence between the ability of drugs to decrease ICSS thresholds and their abuse potential (47).

Many ICSS procedures have been developed over the years, but an important methodologic advance has been the development of procedures that provide a measure of reward threshold that is unconfounded by influences on motor and performance capability. These are the rate-frequency curve-shift procedure, and the discrete-trial, current-intensity procedure (28,47,64). These have been reviewed in detail previously (46) and are not discussed here.

Potential Pitfalls

Brain stimulation reward has the advantage of directly interfacing with brain reward circuits and as such eliminates any interference with consummatory-like behaviors. In addition, it is a validated and reliable measure of brain reward. Potential pitfalls, however, include the requirement for sur-

gery (e.g., the implantation of electrodes). The surgery itself is routine but does require specialized equipment. Another variable in this domain is the brain site selected. Some brain regions support higher rates of brain stimulation reward than others and there may be different circuits activated by different sites.

In addition, animals need to be trained for several weeks to obtain stable rates of responding or stable thresholds. This training requirement and the extensive surgical requirements virtually force the use of within-subject designs. As a result, steps must be taken to address order-effects and analyze such potential confounds.

Animal Models of the Subjective Effects of Drugs: Drug Discrimination

The use of the drug discrimination paradigm in studies of drug addiction is based on two hypotheses. First, the same components of a drug's actions subserve discriminative stimulus effects in animals and subjective effects in humans. Second, discriminative stimulus effects of drugs may contribute to drug taking in intermittent users and to relapse of addiction in former drug addicts. In this latter view, discriminative stimuli signal the availability of a reinforcer and therefore set the occasion to engage in those behaviors that enable consumption of the reinforcing drug. Evidence has been obtained that stimuli predictive of drug administration elicit drug-seeking and -taking behavior and can retard the extinction of responding for psychostimulants (24, 87,97) suggesting that the discriminative stimulus effects of a drug contribute to the genesis of these behaviors.

In a typical experiment, animals are trained to emit a particular response following administration of a fixed drug dose (e.g., depression of one lever designated the drug-associated lever) and to press another lever (saline designated lever) following administration of saline under a fixed schedule of reinforcement. Most commonly, an appetitively motivated operant procedure is used in which animals are food or water deprived. Responding on the training-condition appropriate lever results in the delivery of food or water. Training is continued until the animal reliably selects the appropriate lever after drug or saline administration. Once trained, tests of stimulus generalization or antagonism are implemented to determine whether other doses of the training drug or a specific drug treatment produces stimulus effects qualitatively similar to or different from that of the training drug.

As with other operant paradigms, various reinforcement schedules (e.g., fixed-ratio, fixed-interval, differential reinforcement of low response rate) and response measures (e.g., nose poking, maze running) can be used. Dose 1 versus dose 2 and drug 1 versus drug 2 versus saline discriminations also can be employed. Details can be found in the following references (13,29,46,72).

Reliability and Predictability of Drug Discrimination Procedures

Drug discrimination offers both reliability and predictive validity. The dependent variable is very reliable as a measure of the interoceptive effects of drugs. Stimulus generalization gradients are stable once drug discrimination is acquired and neurotransmitter antagonists alter the stimulus effects of various drugs predictably. Drug discrimination also has predictive validity in that drugs that produce discriminative stimulus effects that generalize to known drugs of abuse have been shown to have abuse liability.

Potential Pitfalls

Generalization gradients are dependent on the dose of drug used for training. Certain neurotransmitter antagonists attenuate the discriminative stimulus effects of a drug when a low training dose is employed. However, these same antagonists fail to modify the discriminative stimulus effects of the same drug when a higher training is employed (41). Similarly, generalization to partial agonists or mixed agonists/antagonists can differ depending on the training dose employed (19); therefore, the use of multiple training doses is essential.

Different test procedures (extinction versus reinforced responding on the lever on which the first schedule requirement is completed) may yield different results depending on the variable used to measure generalization. As with all animal models, species and strain differences as well as the experimental history of an animal can alter the discriminative stimulus effects of a drug (94). Finally, subtle differences in the discriminative stimulus effects of a drug may occur depending on whether appetitive or aversively maintained responding is employed.

ANIMAL MODELS OF THE NEGATIVE REINFORCING EFFECTS OF DRUG WITHDRAWAL

Withdrawal from chronic drug administration usually is characterized by responses opposite to the acute initial actions of the drug. Many of the overt physical signs associated with withdrawal from drugs (e.g., alcohol and opiates) can be quantified easily. However, motivational measures of abstinence have proven to be more sensitive measures of drug withdrawal and powerful tools for exploring the neurobiological bases for the motivational aspects of drug dependence.

Operant Drug Self-Administration

Drug self-administration can be conducted under conditions in which animals are rendered physically dependent

on the drug (e.g., abstinence from drug use results in a withdrawal syndrome), and the procedures are similar to those discussed in the preceding. Although it is clear that animals will self-administer drugs in the absence of withdrawal, some evidence suggests that physical dependence can increase the reinforcing efficacy of a drug. Monkeys made physically dependent on morphine show increases in their progressive-ratio performance compared to animals that do not exhibit withdrawal symptomology (101). Also, baboons in a discrete-trials choice procedure for food and heroin showed significant behavioral plasticity when allowed periodic access to heroin or food (20). In the withdrawal state, one would hypothesize that the animals would be much less likely to respond for food, even if the cost of heroin in terms of response requirements was dramatically increased. Thus, the reinforcing value of drugs may change depending on the presence or absence of a withdrawal state. The neurobiological basis for such a change is only beginning to be investigated, but much evidence has been generated to show that drug withdrawal can produce an aversive or negative motivational state that is manifested by changes in a number of behavioral measures including response disruption, increased drug intake, changes in reward thresholds, and place aversions.

Recent studies with alcohol have shown that rats with a history of self-administration of alcohol will self-administer alcohol during withdrawal in sufficient quantities to prevent withdrawal symptoms and maintain blood alcohol levels above 100 mg% (75). To assess the relationship of withdrawal severity, blood alcohol levels, and alcohol self-administration in dependent and nondependent rats, rats were trained to lever press for 10% alcohol versus water using the saccharin fadeout procedure and subjected to induction of dependence on alcohol (75). Dependent animals allowed to respond for alcohol during a second 12-hour test period showed sustained alcohol intake that maintained blood alcohol levels above 100 mg% throughout the 12-hour period, and a virtual elimination of alcohol withdrawal scores (75) (Fig. 97.1). Animals not allowed access to alcohol during withdrawal on a third test showed a precipitous drop in blood alcohol levels and a dramatic increase in withdrawal scores (75) (Fig. 97.1). These results show that rats will maintain and sustain lever pressing for alcohol during dependence if the animals have a history of lever pressing for alcohol to the point of suppressing alcohol withdrawal and maintaining blood alcohol levels.

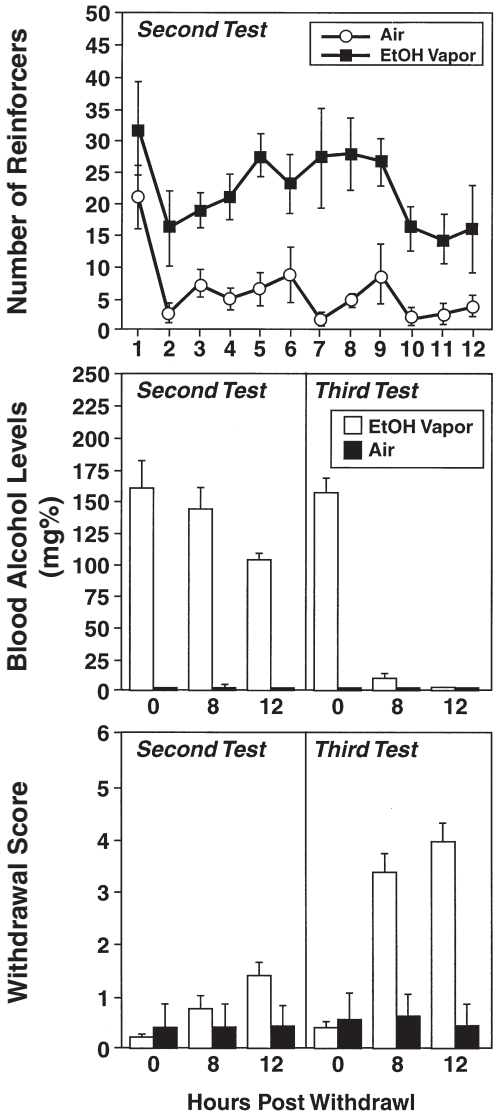

FIGURE 97.1. Operant responding for alcohol across a 12-hour test period by air-exposed and alcohol vapor-exposed rats *(top)*. In addition, blood alcohol levels *(middle)* and alcohol withdrawal severity *(bottom)* obtained during test 2 (while rats were allowed access to alcohol in the operant boxes) and test 3 (while in home cages) are shown. The animals were divided into two groups. One group of animals was assigned to 2 weeks of alcohol exposure in alcohol vapor chambers. The second group was exposed to control air. Rats then were tested in the operant boxes with access to 10% alcohol and water across two 12-hour periods separated by 4 days of vapor exposure. A third and final withdrawal phase was included after another 4 days of vapor exposure; however, animals were kept in their home cages and not allowed to respond for alcohol. Blood was collected for blood alcohol determinations, and observational withdrawal signs were rated during tests 2 and 3 at 0, 8, and 12 hours post withdrawal. Data are expressed as means ± SEM. Taken with permission from Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcohol Clin Exp Res* 1996;20:1289–1298.

Responding for Non-Drug Reinforcers

Several operant schedules have been used to characterize the response-disruptive effects of drug withdrawal (37,84). However, response disruption can be caused by any number of variables from motor problems to malaise and decreases in appetite, and thus other measures must be used to rule out nonspecific effects (see the following).

Conditioned Place Aversion

The conditioned place preference paradigm can also be used to characterize the conditioned aversive effects of drug withdrawal. Rodents are exposed to one environment while undergoing withdrawal and to another in the absence of a withdrawal state. During tests of conditioning, animals are allowed access to both environments and the time spent in each is determined. To date, this procedure has been used almost exclusively to study withdrawal from opiate drugs. Administration of opioid receptor antagonists to animals rendered physically dependent on morphine via implantation of morphine pellets or repeated injections of an opiate produces dose-related conditioned place aversions, an effect that can be observed after only a single conditioning session with the antagonist (27,34). In contrast, the administration of the same doses of antagonist to opiate-naive animals fails to produce a conditioned response. Interestingly, the minimum effective dose of an antagonist that produces conditioned place aversions in animals physically dependent on morphine is less than that producing quantifiable somatic withdrawal signs suggesting that this technique is a particularly sensitive model for evaluating the affective component of drug withdrawal. Although place conditioning typically has been used to characterize antagonist-precipitated withdrawal, more recent work indicates its utility for studies of spontaneous withdrawal (10).

Brain Stimulation Reward

ICSS thresholds have been used to assess changes in systems mediating reinforcement processes during the course of drug dependence. Although no actual negative reinforcement is measured using this technique, it is included in this section because it constitutes a model of the aversive motivational state associated with the negative reinforcement of drug abstinence in dependent animals. Acute administration of psychostimulant drugs lowers ICSS threshold (i.e., increases ICSS reward) (47), and withdrawal from chronic administration of these same drugs elevates ICSS thresholds (i.e., decreases ICSS reward) (22,44,56) (Fig. 97.2). Similar results have been observed with precipitated withdrawal in opiate-dependent rats (82). Rats showed dramatic increases in ICSS thresholds to naloxone injections that occurred in a dose-related manner and at doses below which obvious physical signs of opiate withdrawal were manifest. These doses of naloxone had no effect on reward thresholds in nondependent animals.

Drug Discrimination

Drug discrimination can be used to characterize both specific and nonspecific aspects of withdrawal. Generalization to an opiate antagonist provides a more general nonspecific measure of opiate withdrawal intensity and time course (26, 30). Examples of a more specific aspect of withdrawal are animals that have been trained to discriminate pentylenetetrazol, an anxiogenic-like substance, from saline in alcohol-, diazepam-, and opiate-dependent animals. During withdrawal, generalization to the pentylenetetrazol cue has suggested an anxiogenic-like component to the withdrawal syndrome (14,21).

Ethological Measures

Animals models of withdrawal that illustrate aversive stimulus effects can be extended to observational measures, some of which may be common to withdrawal from many different drugs of abuse. Increased anxiety-like responses are observed following abstinence from cocaine, opiates, benzodiazepines, and alcohol (9,25,79,81,85). Measures used to assess anxiety-like responses during include validated animal models of anxiety such as the elevated plus-maze, light-dark test, defensive withdrawal, and defensive burying.

Advantages and Disadvantages of Animal Models of the Negative Reinforcing Effects of Drugs

The advantages and disadvantages of models used to evaluate drug withdrawal are similar to those described for the positive reinforcing effects of drugs. Clearly, each of the paradigms described has weaknesses, but when combined can provide powerful insights into the motivational effects of drug abstinence.

ANIMAL MODELS OF ESCALATION IN DRUG INTAKE

Animal Models of Sensitization to the Reinforcing Effects of Drugs

Preclinical studies have shown that the repeated intermittent administration of psychostimulants, opiates, and alcohol can result in a long-lasting enhancement of their behavioral effects (92). This phenomenon, referred to as sensitization, has been implicated in the psychosis that occurs in some individuals following repeated psychostimulant use. A role of sensitization in both vulnerability to drug addiction and drug craving has been hypothesized (77). Both self-administration and conditioned place preference procedures have been used to evaluate sensitization to the reinforcing effects of drugs in experimental animals.

Intravenous Self-Administration

In self-administration studies, sensitization to the positive reinforcing effects of drugs is assessed. Typically, animals receive daily, noncontingent injections of a drug or placebo.

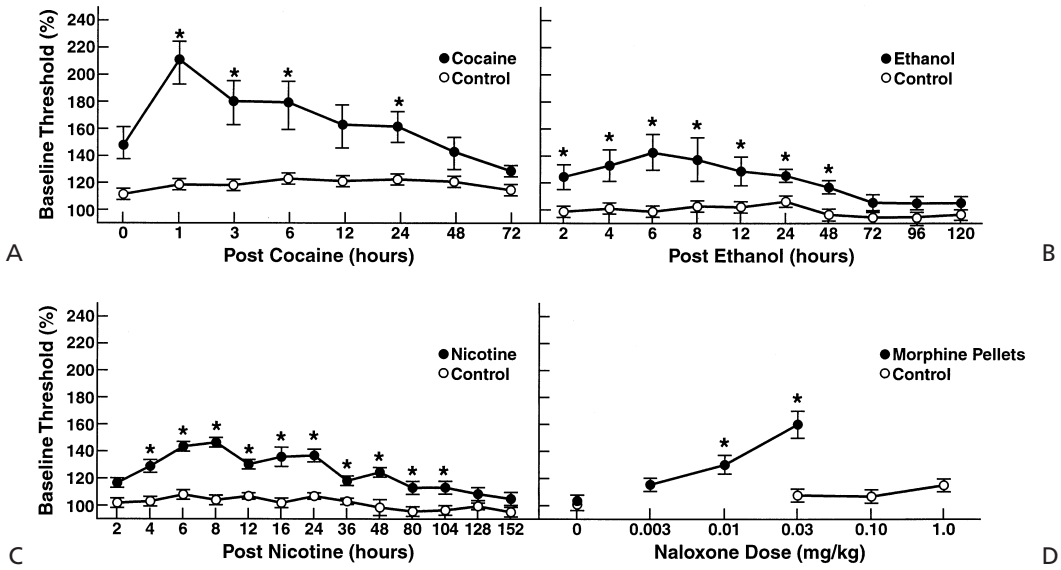

FIGURE 97.2. Changes in reward threshold associated with chronic administration of four major drugs of abuse. Reward thresholds were determined using a rate-independent discrete-trials threshold procedure for intracranial self-stimulation (ICSS) of the medial forebrain bundle. **A:** Rats equipped with intravenous catheters were allowed to self-administer cocaine for 12 straight hours prior to withdrawal and reward threshold determinations. Elevations in threshold were dose-dependent with longer bouts of cocaine self-administration yielding larger and longer-lasting elevations in reward thresholds. Taken with permission from Markou A, Koob GF. Postcocaine anhedonia: an animal model of cocaine withdrawal. *Neuropsychopharmacology* 1991;4:17–26. **B:** Elevations in reward thresholds with the same ICSS technique following chronic exposure to alcohol of approximately 200 mg% in alcohol vapor chambers. Taken with permission from Schulteis G, Markou A, Cole M, et al. Decreased brain reward produced by ethanol withdrawal. *Proc Natl Acad Sci USA* 1995;92:5880–5884. **C:** Elevations in reward thresholds during spontaneous withdrawal after termination of chronic administration of nicotine hydrogen tartrate (9.0 mg/kg per day for 7 days; $n = 8$) or saline ($n = 6$). Taken with permission from Epping-Jordan MP, Watkins SS, Koob GF, Markou A. Dramatic decreases in brain reward function during nicotine withdrawal. *Nature* 1998;393:76–79. **D:** Elevations in reward thresholds following administration of very low doses of the opiate antagonist naloxone to animals made dependent on morphine using two, 75-mg morphine (base) pellets implanted subcutaneously. Taken with permission from Schulteis G, Markou A, Gold LH, et al. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. *J Pharmacol Exp Ther* 1994;271:1391–1398. Asterisks (*) refer to significant differences between treatment and control values. Values are mean ±SEM

Self-administration sessions are then initiated. The rate of acquisition of self-administration and/or the number of animals acquiring stable drug self-administration then is determined. Because sensitization is defined as an increase in the potency and/or efficacy of a drug in producing a particular response following its repeated administration, the rate of acquisition of drug self-administration should be increased and the threshold dose effective in supporting self-administration should be decreased. Several laboratories have shown that the rate of acquisition of psychostimulant self-administration is increased in animals that have received noncontingent injections of these agents indicating the development of sensitization (38,71). The prior administration of amphetamine also increases the acquisition rate of cocaine self-administration (and, conversely, the prior administration of cocaine increases the acquisition rate of amphetamine self-administration), suggesting that cross-sensitization develops to the positive reinforcing effects of psychostimulants.

Conditioned Place Preference

The conditioning procedure used to study sensitization is identical to that described above except that the dose of conditioning drug or the number of environmental pairings used typically are those that are ineffective in producing a conditioned response in previously drug-naive animals. Animals receive repeated noncontingent administration of a drug, and place conditioning can be initiated at various time points following the cessation of drug administration. An increase in the potency of a drug provides a direct measure of sensitization (Fig. 97.3). Alternatively, by employing doses that are subthreshold and threshold for producing a conditioned response, changes in drug potency and efficacy following prior drug exposure can be determined. Using these procedures, long-lasting sensitization and cross-sensitization to the conditioned reinforcing effects of opiates and psychostimulants has been shown (50,52,88).

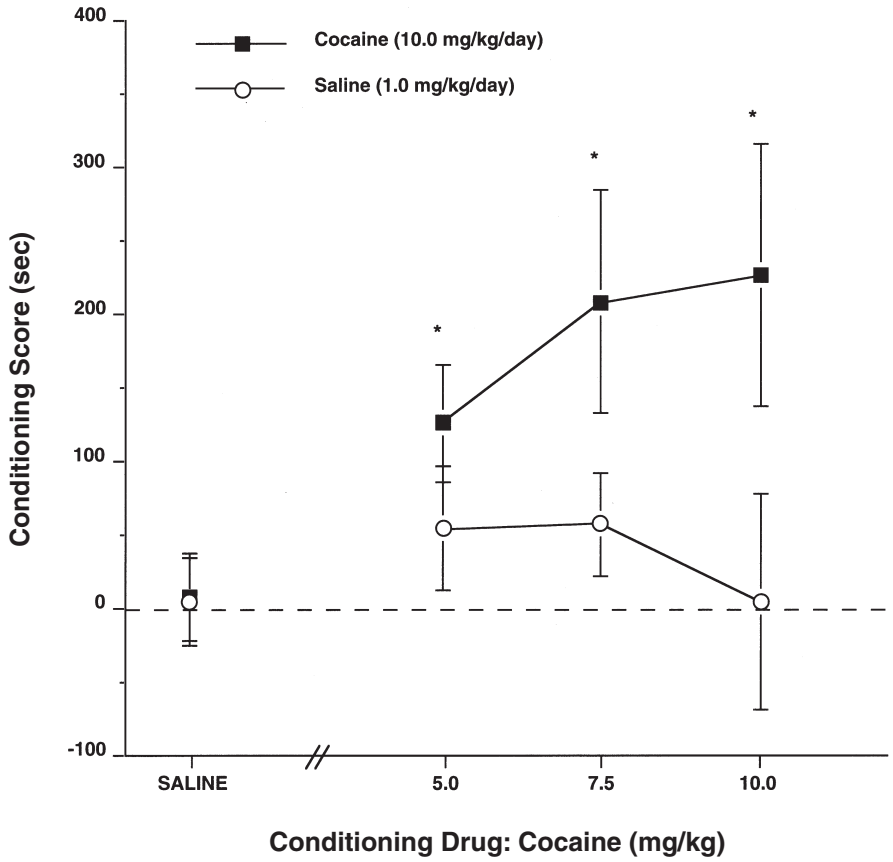

FIGURE 97.3. Sensitization to the conditioned reinforcing effects of cocaine. Rats received once daily home cage injections of cocaine or saline for 5 days. Place conditioning (two cocaine; two saline) commenced 3 days later. Cocaine was ineffective in producing a conditioned response after two environmental pairings. In animals with a prior history of cocaine, doses of cocaine as low as 5.0 mg/kg produced significant conditioned place preferences. Ordinate: Conditioning score defined as time in drug-paired environment minus time in saline-paired environment. Asterisks (*) denote significant place conditioning. Taken with permission from Shippenberg TS, Heidbreder C. Sensitization to the conditioned rewarding effects of cocaine: pharmacologic and temporal characteristics. *J Pharmacol Exp Ther* 1995;273:808–815.

Escalation in Drug Self-Administration Produced by a History of Drug Intake

A critical issue for the study of the neurobiology of addiction is to develop animal models for the transition between controlled/moderate drug intake and uncontrolled/excessive drug intake. Animal models of increased drug intake based on prolonged exposure to drug now have been described in rats for cocaine, heroin, and alcohol (1–3,76).

The pattern of drug self-administration dramatically differs depending on the duration of access. With 1 hour of access to cocaine, drug intake remained at the level of training intake and was stable over time. In contrast, with 6 hours of access per session, cocaine intake gradually escalated to levels significantly above the training baseline (Fig. 97.4). The dose–effect function was shifted up and not to the right or left (2). Abstinence of a month returned the escalated intake to pre-escalation baseline, but escalation was reinstated rapidly at a level higher than that seen before abstinence.

Similar results have been observed in rats trained to self-administer heroin intravenously. Two groups of rats were trained on 1-hour continuous access to intravenous heroin self-administration and then one group was allowed access for 11 hours continuously. In the animals with 11-hour access, intake gradually increased over time, whereas in the animals continued on 1-hour access there was no change in intake over time. The animals with 11-hour access to heroin were slower to extinguish heroin-seeking behavior.

Animals with a history of alcohol exposure sufficient to produce dependence show a similar increase in baseline alcohol intake long after acute withdrawal (76). Operant oral alcohol self-administration was established in rats and then animals were exposed to alcohol vapor chambers for a sufficient period to produce physical dependence on alcohol, detoxified, and then allowed a 2-week period of protracted

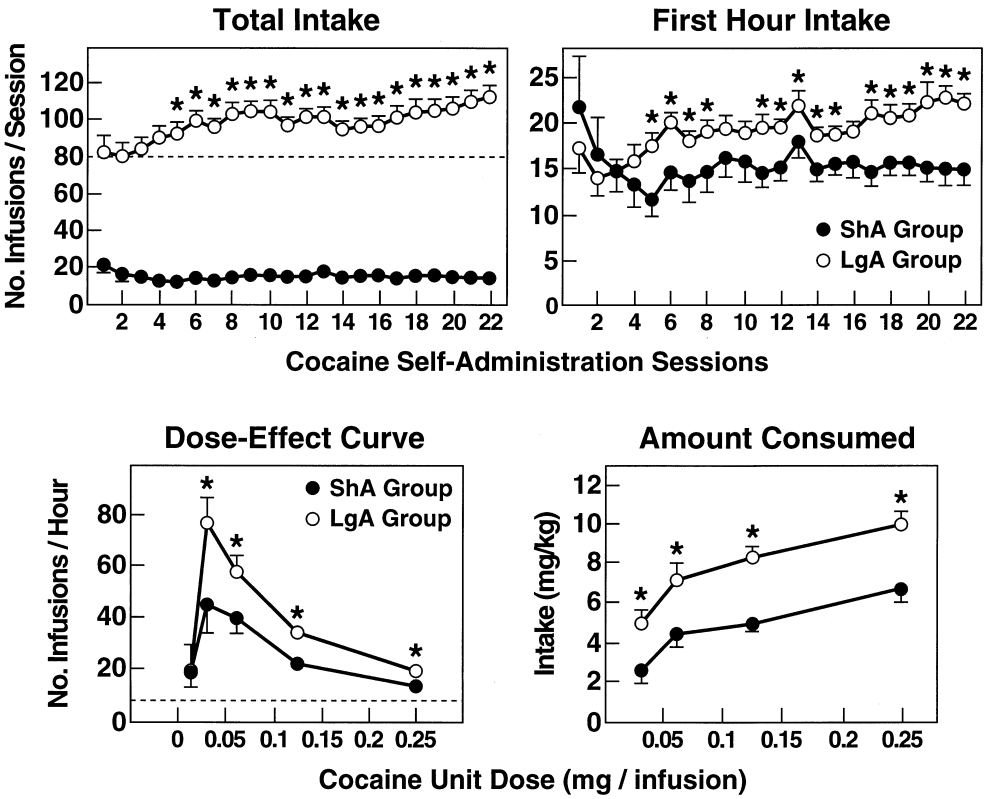

FIGURE 97.4. Reproduction of escalation of cocaine use. **A:** In Long-Access (LgA) rats ($n = 12$) but not in Short-Access (ShA) rats ($n = 12$), mean total cocaine intake ($\pm$SEM) started to increase significantly from session 5 ($p < .05$; sessions 5 to 22 compared to session 1) and continued to increase thereafter ($p < .05$; session 5 compared to sessions 8 to 10, 12, 13, and 17 to 22). **B:** During the first hour, LgA rats self-administered more infusions than ShA rats during sessions 5 to 8, 11, 12, 14, 15, and 17 to 22 ($p < .05$). **C:** Mean infusion ($\pm$SEM) per cocaine dose tested. LgA rats took significantly more infusions than ShA rats at doses of 31.25, 62.5, 125, and 250 μg per infusion ($p < .05$). **D:** After escalation, LgA rats took more cocaine than ShA rats regardless of the dose ($p < .05$). *($p < 0.05$ (Student's *t* test after appropriate one-way and two-way analysis of variance). Taken with permission from Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. *Science* 1998;282:298–300.

abstinence. Operant responding was enhanced during protracted abstinence by 30% to 100% and remained elevated for 4 to 8 weeks post acute withdrawal.

ANIMAL MODELS OF RELAPSE: CONDITIONED REINFORCING EFFECTS OF DRUGS

The role of environmental stimuli in the control of drug-taking behavior is a major focus of addiction research. This interest stems from the view that any account of drug abuse must address those factors that precede and motivate drug taking, as well as those that underlie the reinforcing consequences of drug delivery. Environmental cues repeatedly paired with primary reinforcers can acquire incentive properties via classical conditioning processes (57,87,97). It has been postulated that these conditioned reinforcing effects contribute to drug craving and relapse to addiction. Indeed, human studies have shown that the presentation of stimuli previously associated with drug delivery increases the likelihood of relapse as well as self-reports of craving and motivation to engage in drug taking (17,68).

Positive Reinforcing Effects of Stimuli Associated with Drug Self-Administration: Conditioned Reinforcement Paradigm

The conditioned reinforcement paradigm allows characterization of the incentive value imparted on formerly neutral environmental stimuli that have been repeatedly associated with drug self-administration. In this paradigm, subjects usually are trained in an operant chamber containing two levers. Responses on one lever result in the presentation of a brief stimulus followed by a drug injection (active lever)

whereas responses on the other lever have no consequences (inactive lever) (86). The ability of the previous neutral, drug-paired stimulus to maintain responding in the absence of drug injections provides a measure of the reinforcing value of these stimuli. This procedure provides a stringent test for the conditioned incentive effects of drugs because responding for drug-associated stimuli occurs under extinction conditions (e.g., in the absence of drug). It also provides an animal model of drug craving because the incentive motivational effects of a stimulus are examined in the absence of drug taking.

Second-Order Schedules

Second-order schedules also can be used to evaluate the conditioned reinforcing effects of drugs. To assess the effects of conditioned reinforcement, the number of responses with the paired stimulus can be compared to the number of responses with a nonpaired stimulus. For example, substitution of drug-paired stimuli with nondrug-paired stimuli actually can decrease response rates (43). This maintenance of performance with drug-paired stimuli appears to be analogous to the maintenance and reinstatement of drug seeking in humans with the presentation of drug-paired stimuli (17). In rats, a decrease in responding and an increase in the latency to initiate responding occurs in response to withholding a stimulus paired with cocaine self-administration (8). The schedule can be repeated several times during a test session, resulting in multiple infusions of drug. However, drug craving in the absence of drug can be assessed by terminating sessions immediately after the first drug infusion that occurs after completion of the terminal schedule.

Extinction with and without Cues Associated with Intravenous Drug Self-Administration

Extinction procedures provide measures of the incentive or motivational effects of drugs by assessing the persistence of drug-seeking behavior in the absence of response–contingent drug availability. In this paradigm, subjects first are trained to self-administer a drug until stable self-administration patterns are exhibited. Extinction sessions are identical to training sessions except that no drug is delivered after the completion of the response requirement.

Measures provided by an extinction paradigm reflect the degree of resistance to extinction and include the duration of extinction responding and the total number of responses emitted during the entire extinction session. The probability of reinstating responding under extinction conditions with drug-paired stimuli or even stimuli previously paired with drug withdrawal can be examined.

Both stimulant and opiate self-administration have been consistently reinstated following priming injections of drug (31,55). Responding during extinction is greater in the presence of the conditioned stimulus than in its absence (73). Similar results have been obtained in an operant runway task (57). It is also apparent that environmental stimuli predictive of cocaine self-administration reliably elicit drug-seeking behavior in experimental animals and that responding for these stimuli is highly resistant to extinction (39, 87,97).

Reinstatement of Extinguished Drug-Seeking Behavior in an Animal Model of Relapse: Use of Discriminative Stimuli

Rat models of ''relapse'' induced by drug-related stimuli also can involve the use of a drug-predictive discriminative stimulus (S?). This stimulus is paired with response-contingent presentation of a stimulus that has been contiguously paired with drug presentations (i.e., a conditioned stimulus, or CS) to elicit recovery of responding at a previously active lever after prior extinction of alcohol-seeking behavior. Discriminative stimuli signal the availability of a reinforcer, and thereby provide motivation to engage in behavior that brings the organism into contact with the reinforcer. A condition often associated with drug craving in humans is cognitive awareness of drug availability (63). Discriminative stimuli, therefore, may have a prominent role in craving and the resumption of drug-seeking behavior in abstinent individuals. Moreover, the response-contingent CS, acting as a conditioned reinforcer, may contribute to the maintenance of subsequent drug-seeking behavior once initiated. In fact, these contingencies can be conceptualized to resemble those associated with the relapse process in humans in that certain drug-related cues may provide the initial central motivational state to engage in drug-seeking behavior, whereas others may maintain this behavior until the primary reinforcer is obtained.

To investigate the role of drug-associated stimuli in the motivational effects of a history of cocaine self-administration, rats were trained to associate discriminative stimuli (SΔ) with response-contingent availability of intravenous cocaine versus saline (97) (Fig. 97.5). The rats then were subjected to repeated extinction sessions during which cocaine, saline, and the respective SΔ were withheld until the rats reached extinction. Subsequent re-exposure to the cocaine SΔ, but not the nonreward SΔ, produced strong recovery of responding at the previously active lever in the absence of any further drug availability. The behavioral significance of the cocaine SΔ was further confirmed by the fact that the rats initially tested in the presence of the nonreward SΔ showed complete recovery of responding when subsequently presented with the cocaine SΔ, but rats that had shown robust reinstatement ceased responding when later tested under nonreward SΔ conditions. These results support the hypothesis that learned responses to drug-related environmental stimuli can be important factors in the reinstatement of drug-seeking in animals and provide

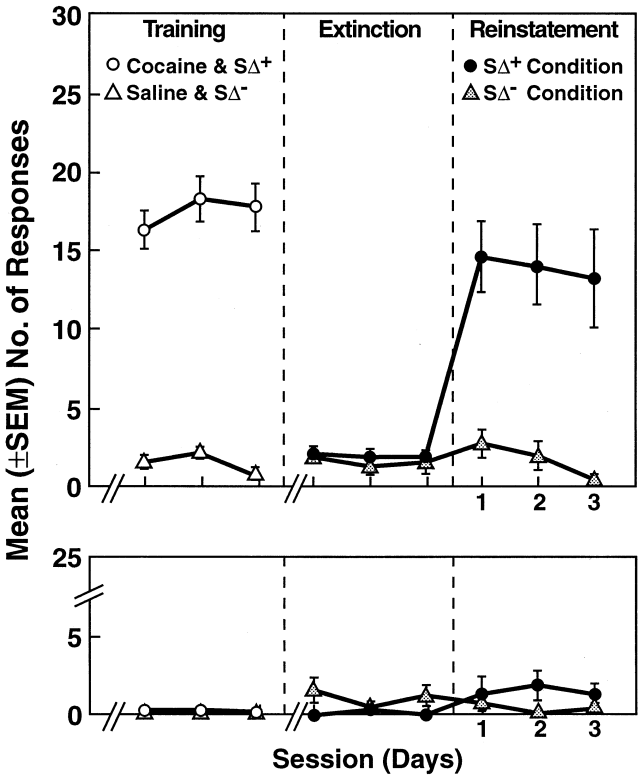

FIGURE 97.5. Lever-press responses during self-administration training, extinction, and reinstatement sessions at the active *(A)* and inactive *(B)* lever. Training phase: cocaine-reinforced responses during the final 3 days of the self-administration phase in rats ($n = 15$) trained to associate S?s with the availability of intravenous cocaine (S^+) versus saline (S^-). No differences were observed between responses during the first and second daily hour of cocaine availability, and responses for cocaine or saline between rats designated for testing under S^+ versus S^- conditions during the initial 3 days of the reinstatement phase. The data were, therefore, collapsed across groups and daily cocaine sessions for the purpose of this illustration. Extinction phase: extinction responses at criterion. The extinction criterion (= 4 responses per session over 3 consecutive days) was reached within 16.4 ± 3.8 days (averaged across rats designated for testing under the S^+ versus S^- condition during the reinstatement phase). Reinstatement phase: responses under the S^+ ($n = 7$) and S^- ($n = 8$) reinstatement conditions. Exposure to the S^+ elicited significant recovery of responding in the absence of further drug availability. Responding in the presence of the S^- remained at extinction levels. Taken with permission from Weiss F, Maldonado-Vlaar CS, Parsons LH, et al. Control of cocaine-seeking behavior by drug-associated stimuli in rats: effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proc Natl Acad Sci USA* 2000;97: 4321–4326.

a powerful model for elucidating the neuropharmacologic basis for such effects that are related to the human concepts of relapse and craving (97).

Cues associated with oral self-administration and availability of alcohol also can reinstate responding in the absence of the primary reinforcer (42,96). In addition, and consistent with the well-established conditioned cue reactivity in human alcoholics, the motivating effects of alcohol-related stimuli are highly resistant to extinction in that they retain their efficacy in eliciting alcohol-seeking behavior over more than 1 month of repeated testing (96).

Place Conditioning

Place conditioning procedures can be modified to serve as a model of relapse. Place aversions to opiate withdrawal last for over 8 weeks (94) and are resistant to extinction. Attempts to modify such conditioned effects could hypothetically contribute to knowledge of the factors that contribute to relapse or "craving." One also could envisage the use of cue- and drug-induced reinstatement of an extinguished place conditioning response as a measure of relapse (67).

Reliability and Predictability

Each of the techniques described has reliability and predictive validity. Presentation of stimuli associated with drug injection induces drug craving in humans and maintains responding in the conditioned reinforcement, second-order schedule, and extinction paradigms. The presence or absence of cues associated with drug administration alters the reinstatement of extinguished drug-seeking behavior in predictable ways.

CONCLUSIONS AND FUTURE RESEARCH

Although it is very difficult to find an animal model of any psychiatric disorder that mimics the entire syndrome, one can reasonably validate animal models for different symptoms of mental disorders (32). In the realm of addiction research, the observation that animals readily self-administer drugs has led to arguments of face validity. Although intravenous drug self-administration meets the criteria of reliability, predictability, and face validity, it does not represent the whole syndrome of addiction (see the following). Other aspects of the addiction syndrome can indeed be modeled, but again, it is incorrect to consider any one of these an animal model of addiction. The DSM-IV criteria for substance dependence and animal models relevant to their study are summarized in Table 97.1.

Tolerance (criterion 1) and withdrawal (criterion 2) no longer define addiction, as illustrated by the change in criteria outlined in DSM-III versus DSM-IIIR and DSM-IV (5–7); however, evidence is accumulating to suggest that a common element associated with addiction is a motivational form of withdrawal that is reflected in a compromised brain reward system (see the preceding). This not only reaffirms the importance of withdrawal in addiction (e.g., criterion 2: "characteristic withdrawal syndrome") but also adds the dimension of a persistent motivational change that may be reflected in criteria 7 of the DSM-IV: "continued use

TABLE 97.1. ANIMAL MODELS FOR THE CRITERIA OF DSM-IV

DSM-IV	Animal Models
A. A maladaptive pattern of substance use, leading to clinically significant impairment or distress as occurring at any time in the same 12-month period:	
(1) Need for markedly increased amounts of substance to achieve intoxication or desired effect; or markedly diminished effect with continued use of the same amount of substance	(1) Tolerance to reinforcing effects: Cocaine Opiates
(2) The characteristic withdrawal syndrome for substance; or substance (or closely related substance) is taken to relieve or avoid withdrawal symptoms	(2) Increased: Reward thresholds: Cocaine, Opiates, Alcohol, Nicotine, Tetrahydrocannabinol Anxiety-like responses: Cocaine, Opiates, Alcohol, Tetrahydrocannabinol
(3) Persistent desire or one or more unsuccessful efforts to cut down or control substance use	(3) Conditional positive reinforcing effects: Cocaine Opiates Alcohol
(4) Substance used in larger amounts or over a longer period than the person intended	(4) Cocaine binge Opiate intake (dependent animals) Alcohol intake (dependent animals) Alcohol Deprivation Effect
(5) Important social, occupational, or recreational activities given up or reduced because of substance use	(5) Choice paradigms Behavioral economics—loss of elasticity
(6) A great deal of time spent in activities necessary to obtain substance, to use substance, or to recover from its effect	(6) Opiate self-administration during withdrawal Alcohol self-administration during withdrawal Progressive-ratio responding
(7) Continued substance use despite knowledge of having a persistent problem that is likely to be caused or exacerbated by substance use	(7) Cocaine binge toxicity

From: American Psychiatric Association, 1994.

despite knowledge of having had a persistent psychological problem that is likely to be exacerbated by the substance'' (Table 97.1).

The two DSM-IV criteria that are best modeled by drug self-administration are criteria 3 and 4, respectively: ''the persistent desire to cut down or control substance use'' and ''the substance taken in larger amounts than intended.'' Drugs of abuse are readily self-administered by animals and, in general, drugs that are self-administered correspond to those that have high abuse potential in humans (60,61).

The chronic relapsing nature of drug addiction is perhaps best illustrated by criterion 3 of Table 97.1: ''persistent desire or one or more unsuccessful attempts to cut down or control substance use.'' Two difficult states to define that are related to relapse are craving and protracted abstinence. Presumably, such states reflect some prolonged post acute withdrawal perturbation or vulnerability to reinstatement of drug-seeking behavior and ultimately compulsive use. A residual deficit state in the reward system or sensitization of the reward system to stimuli that predict drug effects, or some combination of both, could be responsible for this vulnerability (see the preceding).

Animal models of drug craving and relapse continue to be developed but to date have reflected secondary sources of reinforcement such as conditioned reinforcement (91) or residual changes in motivational state or a combination of the two. Second-order schedules can be used as a measure of the conditioned reinforcing properties of drugs (43). Recent work suggests that reliable responding for cocaine can be obtained with a second-order schedule in rats (99). The conditioned place preference paradigm also provides a measure of conditioned reinforcement that is conceptually similar to the measures provided by the operant paradigms. More recently, stimuli that predict drug availability have been shown to be powerful cues for reinstating drug-seeking behavior (97), and a history of drug intake produces escalation in drug intake (1,3).

The remaining criterion for substance dependence as defined by DSM-IV can be linked to animal models by extension to the models described in the preceding. ''Substance taken in large amounts or over a longer period of time than the person intended'' (criterion 4) clearly is reflected in animal models of self-administration with unlimited access, or in situations of limited access where reinforcement value

is challenged by dose–effect functions or progressive-ratio procedures. ''Important social, occupational, or recreational activities given up because of substance use'' (criterion 5) has been demonstrated in animal models involving choice procedures (16) and involving behavioral economics paradigms (20). ''A great deal of time spent in activities necessary to obtain the substance'' (criterion 6) is reflected in animal models of drug self-administration during withdrawal (see the preceding). Finally, ''continued substance use despite knowledge of having a persistent or recurrent physical or psychological problem that is exacerbated by the drug'' (criterion 7) may be reflected in animal models of toxicity associated with chronic drug self-administration such as with cocaine, or prolonged changes in reward thresholds following chronic drug exposure (83).

Drug addiction in humans has been characterized as occurring in several stages, although progress from one stage to the next is not inevitable. The first stage is initiation or acquisition, which may lead to habitual use, physical or psychic dependence, and loss of control. An individual may stop taking a drug at any stage; however, relapse to drug taking after a period of abstinence is common. The extent to which the procedures discussed here model the human condition to the point of reliability and predictive validity requires further assessment.

Animal models of addiction are critical for advances in the study of addiction. Addiction is a chronic relapsing disorder comprised of multiple stages and multiple sources of reinforcement. As discussed, the motivating factors for the development, maintenance, and persistence of drug addiction can be broken down into four major sources of reinforcement: positive reinforcement, negative reinforcement, conditioned positive reinforcement, and conditioned negative reinforcement (100). Much progress has been made in identifying the neuronal substrates for the acute positive reinforcing effects of drugs of abuse. A more recent focus has been on the neuronal substrates for negative reinforcement and the conditioned reinforcing effects that contribute to relapse. The future challenge will be to explore the mechanisms involved in animal models of craving and relapse and to relate these mechanisms to vulnerability to addiction. A major advantage of animal models is in the translation of the human condition to the animal model (face validity) and the translation of the neurobiological measures back to the human condition in order to predict vulnerability (predictive validity).

Perhaps the best example of the translation value of animal models of addiction is the development of medications for the treatment of drug abuse (4,35). The opiate antagonist naltrexone long has been known to block self-administration of alcohol (4,95), and preclinical studies eventually led to the use of naltrexone to successfully prevent relapse in detoxified alcoholics (70).

Animal models are critical for the delineation of genetic and environmental factors that lead to and predict vulnerability to addiction. Context-independent drug administration (e.g., experimenter-administered drugs) can provide information about brain changes associated with a history of drug administration and that alter sensitivity to the effects of a drug. However, drug administration in the context of sensitive and validated animal models provides a much more powerful means of linking drug actions and sensitivities to biological and environmental perturbations. The successful implementation of procedures designed to assess functional genomic activity (e.g., screening for changes in the expression of gene activity) to the study of addiction, will require animal models that are reliable and have predictive validity if they are to contribute to our understanding of the neurobiology of drug addiction.

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