

UPDATE ARTICLE

Animal models of alcohol and drug dependence

Cleopatra S. Planeta

School of Pharmaceutical Sciences, Universidade Estadual Paulista (UNESP), Araraquara, SP, Brazil.

Drug addiction has serious health and social consequences. In the last 50 years, a wide range of techniques have been developed to model specific aspects of drug-taking behaviors and have greatly contributed to the understanding of the neurobiological basis of drug abuse and addiction. In the last two decades, new models have been proposed in an attempt to capture the more genuine aspects of addiction-like behaviors in laboratory animals. The goal of the present review is to provide an overview of the preclinical procedures used to study drug abuse and dependence and describe recent progress that has been made in studying more specific aspects of addictive behavior in animals.

Keywords: Animal model; dependence; addiction; drugs of abuse

Introduction

Drug addiction is an immense social challenge, not only because of its health-related consequences but also because of its socioeconomic and legal impact on society. Addiction is a human phenomenon that cannot be reproduced in a laboratory setting without unavoidable constraints. However, some of the behavioral characteristics of this syndrome can be satisfactorily modeled in laboratory animals. In this way, a wide range of techniques have been developed to model specific aspects of drug-taking behaviors.^{1,2} The possibility of studying these behaviors in animals has contributed to the understanding of the neurobiological basis of drug taking and of the brain systems involved in the reward properties of psychoactive substances. However, the major goal of drug abuse research is to uncover the mechanisms of addiction; thus, in the last two decades, new models have been proposed in an attempt to capture the more genuine aspects of addiction-like behaviors in laboratory animals.²

The goal of the present review is to provide an overview of the preclinical procedures used to study drug abuse and dependence and describe recent progress that has been made in studying more specific aspects of addictive behavior in animals.

Free-choice bottle model

The free-choice bottle model is a non-operant self-administration method restricted to the oral route of administration and most frequently used in alcohol addiction research. This method is noninvasive, technically simple, and uses the route of administration whereby humans consume ethanol. Oral ethanol self-administration methods present face and construct

validity as a model of human alcohol consumption, since subjects can choose whether to drink alcohol as well as the amount ingested over the time of exposure. This model can be used to investigate the short- or long-term consequences of exposure to ethanol, as well as the neurobiological mechanisms related to alcohol abuse and addiction.¹ In addition, these methods can also be useful to prospect pharmacological treatments for prevention of excessive alcohol drinking, which points to their predictive validity.³

Richter & Campbell,⁴ in 1940, were the first to report that laboratory rats voluntarily consume ethanol. They showed that rats allocate their drinking between a water bottle and a bottle containing a dilute ethanol solution, which originated the two-bottle preference test. Alcohol consumption by rodents is commonly assessed by this technique, in which alcohol and water solutions are available in their home cages, with food available ad libitum. Alternatively, animals can have concurrent access to water and several other bottles containing different concentrations of ethanol. The free-choice method, using one or more bottles to offer ethanol, is useful to estimate voluntary and spontaneous intake, as the animal is not forced to drink the liquid.⁵ In general, it has been shown that alcohol consumption increases when a higher number of alternative alcohol solutions are presented.⁶

Measurement of ethanol intake is usually performed by weighing water and ethanol bottles once every 24 hours. Alcohol preference is defined in terms of ethanol intake in g ethanol/kg body weight/day, and percentage of total fluid consumed.⁷ However, the effects of ethanol depend not only on the total amount of ethanol consumed by a rat or mouse within 24 hours but also on the time course and pattern of drinking, measured respectively by the frequency of approaches to an ethanol solution and by the amount consumed per drinking approach.⁸ The use of both criteria is intended to eliminate bias of animals with apparent high alcohol consumption because of small body weight or high fluid intake.⁷

Rodents studied under the condition of continuous access to the solutions generally do not drink enough to attain ethanol blood concentrations above 80 mg/dL (rats) or 100 mg/dL (mice), which can be considered excessive drinking in rats and mice, respectively.^{9,10} It has been shown that ethanol consumption increases with intermittent access. The model of intermittent access (every other 24-hour period) to ethanol in rats led to drinking patterns of high ethanol consumption (9 g/kg/day).¹¹ Much evidence suggests that allowing access to ethanol on an intermittent basis may provide a methodological means of enhancing intake.¹²

Alcohol concentration is another critical issue in these procedures, since low concentrations could be consumed because of their mildly sweet taste and high concentrations rejected because of their aversive taste. Thus, it is usually considered that ethanol concentrations below 4% (v/v) will not create blood concentrations high enough to cause relevant pharmacological effects, and that a concentration in the range of 8-12% is a suitable standard for consumption by rodents. As most rodent strains usually do not drink from highly concentrated ethanol solutions, several procedures have been developed to train rodents to orally self-administer pharmacologically relevant amounts of alcohol, including the presentation of ascending concentrations of ethanol and the restriction of a time period of forced exposure to ethanol.^{1,6}

Another way to increase ethanol consumption involves the manipulation of the incentive value of the solution by increasing its palatability; this can be achieved by adding a sweet flavoring agent, such as sucrose or saccharin, to the ethanol solution. The concentration of the sweetener can be kept constant or progressively decreased over the exposure period.¹²

It is important to note that since the late 1940s, rodent strains have been created by selective breeding for high ethanol preference. Since then, several strains of rats and mice have been selected for high vs. low ethanol preference and used in hundreds of publications in the field of alcohol addiction.¹³

Liquid diet

In the classic study of Lieber & DeCarli,¹⁴ ethanol was added in high concentrations to a liquid diet that was the sole source of nutrition, forcing rats or mice to take the ethanol contained in the diet. The diet was composed in such a way that its nutritional value overcame the aversive gustatory properties of alcohol and produced alcohol intakes of up to 14-16 g/kg/day.

In a more recent study performed by Gilpin et al.,¹⁵ rats were allowed ad libitum access to a 9.2% (v/v) ethanol-liquid diet in which 41% of diet calories were derived from ethanol. The authors showed that the mean daily intake of the 9.2% (v/v) alcohol-liquid diet was 79.04 ± 3.64 mL across all days of the experiment, which was equivalent to an ethanol intake of 9.52 ± 0.27 g/kg/day. The mean resultant blood alcohol concentrations were 352 mg/dL, measured two hours after the beginning of the dark cycle, and near 80 mg/dL 8 hours after the beginning of the light

cycle. Thus, although consumption of the liquid diet is lower during the light phase, rats consumed enough to maintain pharmacologically relevant blood alcohol concentrations. The intake of ethanol during the liquid-diet exposure was also able to elevate operant alcohol responding when rats were tested during withdrawal from the liquid diet.

Besides the ability to produce a specific constellation of somatic withdrawal symptoms in otherwise healthy animals,^{16,17} and enabling study of the reinforcing and motivational properties of ethanol,¹⁵ the technique of feeding alcohol as part of a liquid diet leads to blood alcohol levels that mimic clinical conditions and allows experimental duplications of many pathological complications caused by alcohol, such as alcoholic fatty liver disease, various alcohol-induced metabolic derangements, and the interaction of ethanol with industrial solvents, many commonly used drugs, and nutrients.¹⁸

Alcohol vapor

The alcohol vapor inhalation model was developed in an attempt to induce a state of alcohol dependence.^{19,20} The protocol employs alcohol vapor inhalation systems that are commercially available to expose rats or mice to ethanol vapor. Alcohol vapor inhalation is a noninvasive procedure that allows control of the dose, duration, and pattern of exposure as determined by the experimenter, and is not limited by the predisposition of an animal to voluntarily consume alcohol. Upon cessation of alcohol vapor exposure, animals exhibit signs of tolerance and physical dependence and may be tested for a multitude of motivational, acute withdrawal- and protracted abstinence-related behaviors.²¹

Gilpin et al.¹⁵ exposed rats to alcohol vapor for 4 hours and measured alcohol concentration in brain dialysates and blood samples collected from the tail vein at 30-minute intervals during the 4-hour exposure, as well as 8 hours following termination of alcohol vapor exposure. They found that the maximum levels of alcohol attained in blood and brain during vapor exposure were 208 ± 15 mg/dL and 215 ± 25 mg/dL respectively. Eight hours after cessation of alcohol vapor exposure, blood- and brain-alcohol levels returned to the pre-vapor baseline, approximately 0%.

Gilpin et al.¹⁵ also exposed rats to chronic intermittent alcohol vapor to model the human condition in which alcohol exposure occurs in a series of extended intakes followed by periods of withdrawal. Vapor was delivered on an intermittent schedule (on at 6:00 p.m., off at 8:00 a.m.) for a period of 4 weeks. Chronic exposure to intermittent vapor elicits higher alcohol administration than continuous vapor exposure.²² Blood alcohol levels were assessed via tail vein sampling, and evaporated ethanol values (mL/h) into the vapor chamber were adjusted as necessary to maintain blood levels of alcohol in the 125-250 mg/dL range. The authors employed operant procedures to test the motivational aspects of alcohol dependence. Vapor exposure increased operant responses for 10% w/v oral alcohol when rats were tested

at 6-8 hours of withdrawal during representative post-vapor test days. Previous studies using the chronic intermittent alcohol vapor model showed that motivational symptoms of dependence are present in rats at acute withdrawal time points, as evidenced by increased anxiety-like behavior, increased alcohol drinking, and increased willingness to work for alcohol early during acute withdrawal, even when animals still have alcohol in their blood from vapor exposure.²¹⁻²⁵ All animal models of alcohol dependence are, in fact, models of components of alcohol dependence.

The vapor exposure model has weak face validity, since the animals are forced to consume ethanol. The most interesting aspect of this model is its predictive validity (how well the animal model predicts mechanisms and potential treatments for the human condition). For example, acamprosate, a drug that blocks relapse drinking in human alcoholics via suppression of craving, effectively suppresses alcohol drinking by rats made dependent on alcohol via vapor inhalation, but not in non-dependent controls that had not been exposed to alcohol vapor.²⁶

Operant self-administration

The most direct procedure to evaluate the reinforcing properties of a substance is to test whether animals will work (in general, this means to lever press) to obtain the substance. The use of drug self-administration models to study addiction is based on the assumption that drugs act as reinforcers; that is, they increase the likelihood of the behavior that results in their delivery. Thus, drug self-administration is viewed as an operant response reinforced by the effects of the drug, and it is a common procedure to study voluntary drug intake in laboratory animals. Under this procedure, an animal performs a response, such as pressing a lever, which delivers a dose of a drug. It is assumed that drugs have functional similarities with other reinforcers – such as food – that have traditionally been studied in the field of operant conditioning by Skinner in the 1930s.²⁷

Operant conditioning has been applied as an animal model of drug addiction since the 1960s. Weeks²⁸ described, in 1962, a technique for intravenous self-administration of morphine in the rat. Since then, operant self-administration has been shown for heroin,^{29,30} cocaine,³¹⁻³³ amphetamine,³⁴ nicotine,³⁵⁻³⁷ ethanol,³⁸⁻⁴⁰ and delta-9-THC.⁴¹

Intravenous self-administration is considered the most reliable and predictive experimental model for evaluation of drug-reinforcing effects in animals.²⁷ This method exhibits high face and predictive validity for evaluation of the reinforcing properties of drugs. However, evaluation of the predictive validity of self-administration models for detecting potential therapeutic effects of substances in the treatment of drug addiction is limited by the fact that very few medications are available for this purpose, and, at the present time, are almost completely restricted to alcohol or cigarette smoking.^{1,27}

The apparatus used in conducting an operant drug self-administration procedure consist of commercially available chambers known as operant boxes or Skinner boxes. The chamber has a panel equipped with levers that are pressed by the animal and transmit the response that will activate an infusion pump and deliver a dose of the drug. Other systems based on other responses, such as nose-poking for mice or disk-pecking for pigeons, can be also used. The delivery of the drug can be programmed to match the occurrence of other events, such as lights or tones, as discriminative stimuli and/or secondary reinforcers. The drug is commonly delivered via an intravenous catheter, although other routes can also be used, such as the oral route for ethanol or inhalation for nicotine.^{27,36}

Intravenous self-administration involves surgical implantation of a catheter into the jugular vein. The catheter is passed subcutaneously to the rat's back, where it exits through a small incision and is affixed to a plastic pedestal that can be mounted inside a harness system. After surgery, the animals are allowed to recover for several days in their home cages, with free access to food and water, before the start of the conditioning procedure. A hole in the ceiling of the operant chamber allows the passage and free movement of the tethered catheter, which is connected to a counterbalanced swivel and an infusion pump.^{27,36}

The first phase of this model is acquisition of the operant behavior. To this end, animals are trained in a continuous reinforcement in which each response (lever pressing) is reinforced with the delivery of a infusion of the drug (intravenous self-administration) or a drop of the solution (oral self-administration). The acquisition of drug self-administration is sensitive to environmental and pharmacological manipulations. For example, Covington & Miczek⁴² reported that a significantly greater proportion of rats previously exposed to cocaine (15.0 mg/kg intraperitoneally, once daily for 10 days) acquired cocaine self-administration than control animals that received pretreatment with saline.

In the self-administration paradigm, progressive ratio (PR) schedules are used to assess the motivation to obtain a drug. A PR schedule of reinforcement is implemented through an increase in the number of responses required to obtain the delivery of the drug infusion. For example, Richardson & Roberts⁴³ proposed an algorithm for each successive cocaine infusion in order to produce a series of increasing response demands that would start with a ratio of one and escalate quickly enough so that the rat would not meet a successive response criterion within 60 minutes, during a 5-hour session. The ratio progression was 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178... The last completed ratio, which results in the final infusion, is defined as the breaking point. In the self-administration protocol, the breaking point under PR schedules reflects the motivation of the animal to self-administer the drug.

Recently, we used the PR schedule to assess possible elevations in the breaking point for provision of intravenous nicotine in animals pre-exposed to variable stress.

After the acquisition and maintenance phase, self-administration according to a PR schedule of drug reinforcement was assessed. The progression of response requirements followed the algorithm 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26... Rats had 60 minutes to successfully complete each ratio requirement. The final infusion delivered was defined as the breaking point.^{36,37} In our study, PR schedules revealed a significant increase in breaking points in rats pre-exposed to stress relative to controls, suggesting that exposure to stress can increase motivation for nicotine self-administration. These data are consistent with other findings showing that exposure to four episodes of defeat stress increases the cocaine breaking point during a PR schedule.⁴² Similarly, it has been demonstrated that rats exposed to foot-shock stress had increased PR breaking points for heroin relative to their controls.⁴⁴

The self-administration protocol can be also used to measure the reinforcing effects of drugs under conditions of prolonged access (commonly 24 hours) in a continuous reinforcement schedule, which is known as a binge. Results from our laboratory showed that pretreatment with cocaine increased nicotine intake in a 24-hour binge session of intravenous nicotine self-administration.³⁷

The main disadvantage of self-administration procedures is that they are time-consuming and relatively expensive in comparison with other methods. In addition, long-term studies using the intravenous route in rodents are limited by the duration of the implanted catheters.²⁷

Place conditioning

In the conditioned preference procedure, the effects of the drug, which are presumed to act mainly as the unconditioned stimulus (US), are repeatedly paired with a previously neutral stimulus. In this process, which is Pavlovian in nature, the neutral stimulus acquires the ability to act as a conditioned stimulus (CS). Thereafter, this CS will be able to elicit approach behavior when the drug has appetitive properties. The most common methods used to study conditioned preference apply an environmental stimulus as the CS and are referred to as conditioned place preference (CPP). The testing apparatus for the CPP paradigm usually consists of boxes with two distinct compartments, separated by guillotine doors, which differ in the stimulus dimensions. For example, the compartments may differ in flooring, wall color, pattern, or olfactory cues.⁴⁵ A third (neutral) compartment that will not be paired with the drug is also commonly present in the apparatus.⁴⁶

A typical CPP protocol consists of three phases: pre-conditioning, conditioning, and post-conditioning (test). In the pre-conditioning phase, each animal (rat or mouse) is placed in the neutral compartment with the guillotine doors removed to allow access to the entire apparatus for 15 minutes for 3 days. On day 3, the animal is placed in the apparatus and the time spent in each compartment is recorded. For the conditioning phase, the compartments are isolated by the guillotine doors and the same animal receives alternate injections of the drug and its vehicle. The

drug injection is paired with a specific compartment and the vehicle injection with the alternative one. Immediately after each injection, the animal is confined for 30-40 minutes in the corresponding compartment. For the conditioning test, the animal is placed in the neutral compartment with the guillotine doors removed to allow access to the entire apparatus. The time spent in each compartment is recorded for 15 minutes as described for the pre-conditioning phase; the test is performed in a drug-free condition.⁴⁶ An increase in the time spent in the compartment paired with the effect of the drug indicates the development of CPP and, thus, the appetitive effect of the drug.

CPP has been reported to all drugs that cause dependence in humans; however, results are more robust for opiates and psychostimulants.⁴⁵

Animal studies of addictive behavior

The use of the models described above has significantly increased our comprehension of the neurobiological basis of drug taking. However, the main purpose of drug abuse research is to focus on the mechanisms of addiction. Addiction is not just the taking of drugs, but the maintenance of compulsive drug use despite adverse consequences. The loss of control results in higher drug consumption, in compulsive drug seeking, and in inability to abstain from its use. Thus, in recent years, great efforts have been made to use the self-administration method to model more specific elements of addictive behavior as opposed to merely investigate drug reinforcement. In particular, efforts have been directed to identify whether the DSM-IV criteria for diagnosis of drug addiction can be modeled in an animal.²

The landmark study of Deroche-Gamonet et al.⁴⁷ is an example of this new strategy for investigation of drug addiction. The authors used intravenous self-administration of cocaine to investigate whether addiction-like behaviors could be observed in rodents. They showed that behaviors that resemble three of the essential diagnostic criteria for addiction (difficulty stopping or limiting drug intake; extremely high motivation to take the drug, with activities focused on its procurement and consumption; and continued substance use despite its adverse consequences) can be modeled in rats trained to self-administer cocaine.

Escalation of drug use is characteristic of the transition from occasional drug use to addiction. Long extended access (binge, see above) has been widely used to demonstrate the escalation of drug intake, especially of cocaine and ethanol. Rats with extended access to drug self-administration gradually increase their intake over the course of days, in a manner that is not directly related to tolerance. For example, rats with extended access (6 hours/day) to cocaine self-administration gradually increased their cocaine intake across days, whereas those with limited drug access (1 hour/day) maintained remarkably stable rates of drug self-administration, even after several months of testing.^{48,49} The escalation of cocaine intake with extended access to the self-administered drug has been reported in several reports.⁵⁰⁻⁵² Rats that

displayed escalated cocaine self-administration also showed increased motivation for the drug, as evidenced by increased breaking points in PR schedules,⁵³ which models another behavioral characteristic of addictive behavior.

Compulsive drug use despite adverse consequences has also been modeled in preclinical studies. In these studies, the behavior of seeking or taking drugs was paired with a negative stimulus. For example, Vanderschuren et al.⁵⁴ showed that pairing an aversive CS (foot shock) with cocaine self-administration suppressed drug-seeking behavior in rats with limited cocaine self-administration experience, but not in rats that had had previous prolonged access to cocaine taking.

In studies using oral ingestion of drugs, especially ethanol, the intake of a solution containing bitter-tasting quinine is commonly used as the aversive stimulus.⁵⁵ The addition of quinine to an ethanol solution that had been previously available to rats for 3–4 months did not reduce their intake of ethanol despite the bitter taste of quinine.⁵⁶ Similarly, Lesscher et al.⁵⁷ reported that mice became indifferent to quinine after prolonged access (8 weeks) to ethanol, as they drank equal amounts of ethanol from bottles with and without quinine at an aversive concentration.

Difficulty in abstaining from drug use is also characteristic of drug addiction; this can be studied in laboratory animals by assessing drug seeking in the self-administration model when the drug is no longer delivered in response to a lever press by the animal. This resistance to extinction of the operant behavior has been observed in rats with a history of extended access to heroin or cocaine self-administration.^{47,58}

Addiction has characteristics of a chronic relapsing disorder. Indeed, a significant number of addicted individuals relapse to drug-taking even after a prolonged period of withdrawal; thus, a preclinical model for relapse is also important in the study of the mechanisms of addiction. In this sense, de Wit & Stewart⁵⁹ reported that non-contingent priming injections of cocaine or re-exposure to cocaine-paired cues reinstated lever-pressing behavior following extinction of the operant response. Based on these results, they suggested that their reinstatement model could be used to study factors involved in drug use relapse.

Two animal models have proven especially useful for studying relapse.⁶⁰ One is reinstatement of self-administration.^{61,62} The second experimental model to study relapse in animals is the reinstatement of CPP.^{46,63,64} In these models, animals are first trained to acquire the conditioned response, and then undergo a process of extinction of this behavior. Once the behavior is extinguished, experimental manipulations (i.e., contingent exposure to drug or non-drug stimuli) are imposed and lead to the resumption of a previously drug-reinforced behavior. The apparent similarity of this outcome and relapse has led to the use of this procedure as a model of relapse and as an assessment of craving.⁶⁰

A relevant aspect of the reinstatement model is the observation that factors which provoke relapse and

craving in humans are also reported to reinstate drug seeking in laboratory animals. These factors include re-exposure to the drug or drug-associated cues and exposure to stressors.^{65,66}

Exposure to stressful events is considered a major factor responsible for drug relapse.^{67,68} Preclinical studies have shown that stress can reinstate nicotine, cocaine, heroin, and ethanol self-administration.⁶⁹⁻⁷¹ Similarly, several studies have shown that stress exposure induces the reinstatement of opioid-, amphetamine-, cocaine-, and nicotine-induced CPP.^{64,71-74}

There is reasonable evidence to support the face validity of the reinstatement model, but neither its predictive validity nor its functional equivalence have been fully established.⁶⁰

Concluding remarks

This review has summarized some procedures commonly used for the evaluation of abuse and dependence liability. These animal models are widely employed to study the neurobiological and molecular mechanisms of drug taking. Moreover, recent advances in modeling the symptoms of addiction in animal studies, based on the DSM-IV criteria, present an exciting opportunity for study of the neural and genetic background of drug addiction. These new approaches are also excellent tools for the investigation of therapeutic agents to improve coping strategies in the addicted patient.

Acknowledgements

Cleopatra S. Planeta is a research fellow of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Disclosure

The authors report no conflicts of interest.

References

- 1 Sanchis-Segura C, Spanagel R. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. *Addict Biol.* 2006;11:2-38.
- 2 Vanderschuren LJ, Ahmed SH. Animal studies of addictive behavior. *Cold Spring Harb Perspect Med.* 2013;3:a011932.
- 3 Spanagel R, Zieglgansberger W. Anti-craving compounds for ethanol: new pharmacological tools to study addictive processes. *Trends Pharmacol Sci.* 1997;18:54-9.
- 4 Richter CP, Campbell KH. Alcohol Taste Thresholds and Concentrations of Solution Preferred by Rats. *Science.* 1940;9:507-8.
- 5 Tordoff MG, Bachmanov AA. Influence of the number of alcohol and water bottles on murine alcohol intake. *Alcohol Clin Exp Res.* 2003;27:600-6.
- 6 Boyle AE, Smith BR, Spivak K, Amit Z. Voluntary ethanol consumption in rats: the importance of the exposure paradigm in determining final intake outcome. *Behav Pharmacol.* 1994;5:502-12.
- 7 McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol.* 1998;12:339-69.
- 8 Leeman RF, Heilig M, Cunningham CL, Stephens DN, Duka T, O'Malley SS. Ethanol consumption: how should we measure it?

- Achieving consilience between human and animal phenotypes. *Addict Biol.* 2010;15:109-24.
- 9 Bell RL, Rodd ZA, Lumeng L, Murphy JM, McBride WJ. The alcohol-preferring P rat and animal models of excessive alcohol drinking. *Addict Biol.* 2006;11:270-88.
 - 10 Crabbe JC, Metten P, Rhodes JS, Yu CH, Brown LL, Phillips TJ, et al. A line of mice selected for high blood ethanol concentrations shows drinking in the dark to intoxication. *Biol Psychiatry.* 2009;65:662-70.
 - 11 Wise RA. Voluntary ethanol intake in rats following exposure to ethanol on various schedules. *Psychopharmacologia.* 1973;29:203-10.
 - 12 Crabbe JC, Harris RA, Koob GF. Preclinical studies of alcohol binge drinking. *Ann N Y Acad Sci.* 2011;1216:24-40.
 - 13 Crabbe JC, Phillips TJ, Belknap JK. The complexity of alcohol drinking: studies in rodent genetic models. *Behav Genet.* 2010;40:737-50.
 - 14 Lieber CS, De Carli LM. Ethanol dependence and tolerance: a nutritionally controlled experimental model in the rat. *Res Commun Chem Pathol Pharmacol.* 1973;6:983-91.
 - 15 Gilpin NW, Smith AD, Cole M, Weiss F, Koob GF, Richardson HN. Operant behavior and alcohol levels in blood and brain of alcohol-dependent rats. *Alcohol Clin Exp Res.* 2009;33:2113-23.
 - 16 Frye GD, Chapin RE, Vogel RA, Mailman RB, Kilts CD, Mueller RA, et al. Effects of acute and chronic 1,3-butanediol treatment on central nervous system function: a comparison with ethanol. *J Pharmacol Exp Ther.* 1981;216:306-14.
 - 17 Majchrowicz E. Induction of physical dependence upon ethanol and the associated behavioral changes in rats. *Psychopharmacologia.* 1975;43:245-54.
 - 18 Lieber CS, DeCarli LM. Recommended amounts of nutrients do not abate the toxic effects of an alcohol dose that sustains significant blood levels of ethanol. *J Nutr.* 1989;119:2038-40.
 - 19 Goldstein DB, Pal N. Alcohol dependence produced in mice by inhalation of ethanol: grading the withdrawal reaction. *Science.* 1971;172:288-90.
 - 20 Rogers J, Wiener SG, Bloom FE. Long-term ethanol administration methods for rats: advantages of inhalation over intubation or liquid diets. *Behav Neural Biol.* 1979;27:466-86.
 - 21 Gilpin NW, Richardson HN, Cole M, Koob GF. Vapor inhalation of alcohol in rats. *Curr Protoc Neurosci.* 2008;Chapter 9:Unit 9.29.
 - 22 O'Dell LE, Roberts AJ, Smith RT, Koob GF. Enhanced alcohol self-administration after intermittent versus continuous alcohol vapor exposure. *Alcohol Clin Exp Res.* 2004;28:1676-82.
 - 23 Funk CK, Zorrilla EP, Lee MJ, Rice KC, Koob GF. Corticotropin-releasing factor 1 antagonists selectively reduce ethanol self-administration in ethanol-dependent rats. *Biol Psychiatry.* 2007;61:78-86.
 - 24 Roberts AJ, Cole M, Koob GF. Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcohol Clin Exp Res.* 1996;20:1289-98.
 - 25 Valdez GR, Roberts AJ, Chan K, Davis H, Brennan M, Zorrilla EP, et al. Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: regulation by corticotropin-releasing factor. *Alcohol Clin Exp Res.* 2002;26:1494-501.
 - 26 Le Magnen J, Tran G, Durlach J, Martin C. Dose-dependent suppression of the high alcohol intake of chronically intoxicated rats by Ca-acetyl homotaurinate. *Alcohol.* 1987;4:97-102.
 - 27 Panlilio LV, Goldberg SR. Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction.* 2007;102:1863-70.
 - 28 Weeks JR. Experimental morphine addiction: method for automatic intravenous injections in unrestrained rats. *Science.* 1962;138:143-4.
 - 29 Bonese KF, Wainer BH, Fitch FW, Rothberg RM, Schuster CR. Changes in heroin self-administration by a rhesus monkey after morphine immunisation. *Nature.* 1974;252:708-10.
 - 30 Pattison LP, McIntosh S, Budygin EA, Hemby SE. Differential regulation of accumbal dopamine transmission in rats following cocaine, heroin and speedball self-administration. *J Neurochem.* 2012;122:138-46.
 - 31 Hill SY, Powell BJ. Cocaine and morphine self-administration: effects of differential rearing. *Pharmacol Biochem Behav.* 1976;5:701-4.
 - 32 Miczek KA, Mutschler NH. Activational effects of social stress on IV cocaine self-administration in rats. *Psychopharmacology (Berl).* 1996;128:256-64.
 - 33 Cruz FC, Quadros IM, Hogenelst K, Planeta CS, Miczek KA. Social defeat stress in rats: escalation of cocaine and "speedball" binge self-administration, but not heroin. *Psychopharmacology (Berl).* 2011;215:165-75.
 - 34 Pickens R, Harris WC. Self-administration of d-amphetamine by rats. *Psychopharmacologia.* 1968;12:158-63.
 - 35 Goldberg SR, Spealman RD, Goldberg DM. Persistent behavior at high rates maintained by intravenous self-administration of nicotine. *Science.* 1981;214:573-5.
 - 36 Leao RM, Cruz FC, Marin MT, Planeta Cda S. Stress induces behavioral sensitization, increases nicotine-seeking behavior and leads to a decrease of CREB in the nucleus accumbens. *Pharmacol Biochem Behav.* 2012;101:434-42.
 - 37 Leao RM, Cruz FC, Carneiro-de-Oliveira PE, Rossetto DB, Valentini SR, Zanelli CF, et al. Enhanced nicotine-seeking behavior following pre-exposure to repeated cocaine is accompanied by changes in BDNF in the nucleus accumbens of rats. *Pharmacol Biochem Behav.* 2013;104:169-76.
 - 38 Smith SG, Davis WM. Intravenous alcohol self-administration in the rat. *Pharmacol Res Commun.* 1974;6:379-402.
 - 39 Grant KA, Samson HH. Oral self administration of ethanol in free feeding rats. *Alcohol.* 1985;2:317-21.
 - 40 Roberts AJ, Heyser CJ, Koob GF. Operant self-administration of sweetened versus unsweetened ethanol: effects on blood alcohol levels. *Alcohol Clin Exp Res.* 1999;23:1151-7.
 - 41 Justinova Z, Tanda G, Redhi GH, Goldberg SR. Self-administration of delta9-tetrahydrocannabinol (THC) by drug naive squirrel monkeys. *Psychopharmacology (Berl).* 2003;169:135-40.
 - 42 Covington 3rd HE, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology (Berl).* 2001;158:388-98.
 - 43 Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *J Neurosci Methods.* 1996;66:1-11.
 - 44 Shaham Y, Stewart J. Exposure to mild stress enhances the reinforcing efficacy of intravenous heroin self-administration in rats. *Psychopharmacology (Berl).* 1994;114:523-7.
 - 45 Bardo MT, Bevins RA. Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology (Berl).* 2000;153:31-43.
 - 46 Cruz FC, Leao RM, Marin MT, Planeta CS. Stress-induced reinstatement of amphetamine-conditioned place preference and changes in tyrosine hydroxylase in the nucleus accumbens in adolescent rats. *Pharmacol Biochem Behav.* 2010;96:160-5.
 - 47 Deroche-Gamonet V, Belin D, Piazza PV. Evidence for addiction-like behavior in the rat. *Science.* 2004;305:1014-7.
 - 48 Ahmed SH, Koob GF. Transition from moderate to excessive drug intake: change in hedonic set point. *Science.* 1998;282:298-300.
 - 49 Ahmed SH, Koob GF. Long-lasting increase in the set point for cocaine self-administration after escalation in rats. *Psychopharmacology (Berl).* 1999;146:303-12.
 - 50 Ben-Shahar O, Posthumus EJ, Waldroup SA, Ettenberg A. Heightened drug-seeking motivation following extended daily access to self-administered cocaine. *Prog Neuropsychopharmacol Biol Psychiatry.* 2008;32:863-9.
 - 51 Quadros IM, Miczek KA. Two modes of intense cocaine bingeing: increased persistence after social defeat stress and increased rate of intake due to extended access conditions in rats. *Psychopharmacology (Berl).* 2009;206:109-20.
 - 52 Hao Y, Martin-Fardon R, Weiss F. Behavioral and functional evidence of metabotropic glutamate receptor 2/3 and metabotropic glutamate receptor 5 dysregulation in cocaine-escalated rats: factor in the transition to dependence. *Biol Psychiatry.* 2010;68:240-8.
 - 53 Liu Y, Roberts DC, Morgan D. Effects of extended-access self-administration and deprivation on breakpoints maintained by cocaine in rats. *Psychopharmacology (Berl).* 2005;179:644-51.
 - 54 Vanderschuren LJ, Everitt BJ. Drug seeking becomes compulsive after prolonged cocaine self-administration. *Science.* 2004;305:1017-9.

- 55 Wolffgramm J. An ethopharmacological approach to the development of drug addiction. *Neurosci Biobehav Rev.* 1991;15:515-9.
- 56 Hopf FW, Chang SJ, Sparta DR, Bowers MS, Bonci A. Motivation for alcohol becomes resistant to quinine adulteration after 3 to 4 months of intermittent alcohol self-administration. *Alcohol Clin Exp Res.* 2010;34:1565-73.
- 57 Lesscher HMB, Van Kerkhof LWM, Vanderschuren LJM. Inflexible and indifferent alcohol drinking in male mice. *Alcohol Clin Exp Res.* 2010;34:1219-25.
- 58 Ahmed SH, Walker JR, Koob GF. Persistent increase in the motivation to take heroin in rats with a history of drug escalation. *Neuropsychopharmacology.* 2000;22:413-21.
- 59 de Wit H, Stewart J. Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology (Berl).* 1981;75:134-43.
- 60 Katz J, Higgins S. The validity of the reinstatement model of craving and relapse to drug use. *Psychopharmacology (Berl).* 2003;168:21-30.
- 61 Shaham Y, Rajabi H, Stewart J. Relapse to heroin-seeking in rats under opioid maintenance: the effects of stress, heroin priming, and withdrawal. *J Neurosci.* 1996;16:1957-63.
- 62 Shaham Y, Adamson LK, Grocki S, Corrigall WA. Reinstatement and spontaneous recovery of nicotine seeking in rats. *Psychopharmacology (Berl).* 1997;130:396-403.
- 63 Mueller D, Stewart J. Cocaine-induced conditioned place preference: reinstatement by priming injections of cocaine after extinction. *Behav Brain Res.* 2000;115:39-47.
- 64 Ribeiro Do Couto B, Aguilar MA, Manzanedo C, Rodriguez-Arias M, Armario A, Minarro J. Social stress is as effective as physical stress in reinstating morphine-induced place preference in mice. *Psychopharmacology (Berl).* 2006;185:459-70.
- 65 Chiamulera C, Borgo C, Falchetto S, Valerio E, Tessari M. Nicotine reinstatement of nicotine self-administration after long-term extinction. *Psychopharmacology (Berl).* 1996;127:102-7.
- 66 Aguilar MA, Rodriguez-Arias M, Minarro J. Neurobiological mechanisms of the reinstatement of drug-conditioned place preference. *Brain Res Rev.* 2009;59:253-77.
- 67 Sinha R. How does stress increase risk of drug abuse and relapse? *Psychopharmacology (Berl).* 2001;158:343-59.
- 68 Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ. Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. *Arch Gen Psychiatry.* 2006;63:324-31.
- 69 Buczek Y, Le AD, Wang A, Stewart J, Shaham Y. Stress reinstates nicotine seeking but not sucrose solution seeking in rats. *Psychopharmacology (Berl).* 1999;144:183-8.
- 70 Shaham Y, Erb S, Stewart J. Stress-induced relapse to heroin and cocaine seeking in rats: a review. *Brain Res Brain Res Rev.* 2000;33:13-33.
- 71 Schank JR, Pickens CL, Rowe KE, Cheng K, Thorsell A, Rice KC, et al. Stress-induced reinstatement of alcohol-seeking in rats is selectively suppressed by the neurokinin 1 (NK1) antagonist L822429. *Psychopharmacology (Berl).* 2011;218:111-9.
- 72 Cruz FC, Marin MT, Planeta CS. The reinstatement of amphetamine-induced place preference is long-lasting and related to decreased expression of AMPA receptors in the nucleus accumbens. *Neuroscience.* 2008;151:313-9.
- 73 Redila VA, Chavkin C. Stress-induced reinstatement of cocaine seeking is mediated by the kappa opioid system. *Psychopharmacology (Berl).* 2008;200:59-70.
- 74 Leao RM, Cruz FC, Planeta CS. Exposure to acute restraint stress reinstates nicotine-induced place preference in rats. *Behav Pharmacol.* 2009;20:109-13.