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# Principles of drug abuse liability assessment in laboratory animals<sup>☆</sup>

Nancy A. Ator<sup>\*</sup>, Roland R. Griffiths

*Department of Psychiatry and Behavioral Sciences (NAA, RRG), Department of Neuroscience (RRG), Behavioral Biology Research Center, Johns Hopkins School of Medicine, Johns Hopkins Bayview Campus, 5510 Nathan Shock Drive, Ste. 3000, Baltimore, MD 21224-6823, USA*

## Abstract

This paper describes the rationale for use of preclinical assessments of abuse liability in laboratory animals, and then discusses ‘cross-cutting’ methodological issues that apply to behavioral evaluations intended to contribute to an abuse liability evaluation package. Issues include use of: (1) positive and negative control conditions; (2) full dose–effect evaluations, (3) multiple dependent measures, (4) pharmacokinetic evaluations to guide choice of dose ranges, (5) a species for which good methodological and comparative data are available to aid interpretation of results, and (6) appropriate methods for the group or single-subject experimental design selected. The remainder of the paper describes basic methodology by which three core pieces of behavioral data required by the Food and Drug Administration for its use in the overall abuse liability analysis can be obtained preclinically. *Reinforcing* effects are assessed in study of drug self-administration; *drug discrimination* assesses degree of overlap of interoceptive stimulus effects with relevant comparison drugs; *physical dependence potential* is determined by assessing whether a withdrawal syndrome occurs after chronic drug administration. Background and methodological issues specific to each procedure are discussed. A key consideration for cross-cutting and specific methodological issues is that choices made enable confident interpretation of both positive and negative results.

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## 1. Introduction

Abuse liability assessment in laboratory animals is an important component of drug development and evaluation of a psychoactive drug for governmental controls. Studies in laboratory animals have unique advantages over studies in humans. They can be done earlier in an evaluative process, across a greater dose range, and for a more extended duration than in patients or research volunteers.

At early phases of drug development, abuse liability assessment in laboratory animals can contribute to strategic decisions on how and whether to proceed with a new series of centrally acting compounds. If a goal is that the candidate compound be as efficacious as

existing medications but less subject to legal controls on prescribing, then an abuse liability assessment early in drug development can be critical. A comprehensive abuse liability evaluation also contributes to the pharmacological characterization of the compound. For example, such data can show whether a novel molecular mechanism of action results in a novel behavioral profile in comparison to other compounds with the same intended use.

The Food and Drug Administration (FDA), which reviews data relevant to the abuse liability of new centrally acting drugs, expressed the view that ‘the integration of specialized preclinical and clinical abuse liability studies with data [on] safety and efficacy provides the best predictor of the abuse potential of a new drug that has not been marketed anywhere in the world.’ (‘Guidelines for Research Involving the Abuse Liability Assessment of New Drugs’, Draft 12-2-98, FDA Center for Drug Evaluation and Research; Division of Anesthetic, Critical Care and Addiction Drug Products; cf. 1990 Guidelines in Balster and Bigelow, 2003, this volume).

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<sup>\*</sup> Corresponding author. Tel.: +1-410-550-2773; fax: +1-410-550-2780.

E-mail address: ator@jhmi.edu (N.A. Ator).

Similarly the Drug Enforcement Administration (DEA) must evaluate whether compounds that begin appearing in drug abuse trafficking and emergency room overdose cases should be subject to new or increased legal control. Data from abuse liability evaluations are needed to inform final scheduling under the Controlled Substances Act. Due to the nature of some compounds of concern, preclinical studies may be the only ones ethically or practically feasible for such decision making (e.g. chemical analogs of phencyclidine, PCP, Lukas et al., 1984).

Beyond governmental requirements, however, a compelling reason to obtain a comprehensive abuse liability assessment in the process of new drug development is to be able to supply practitioners and patients with information they need for appropriate use. Some caveats accompany use of virtually any efficacious psychoactive drug. The Valium scare of the 70s, which led to Congressional hearings on the benzodiazepines, provides dramatic testimony to the unfortunate consequences of failure to obtain and disseminate data on the abuse liability and physical dependence potential of a clinically useful drug (*Use and misuse of benzodiazepines*, 1979). The belief of physicians and patients that these drugs were completely 'safe' in this regard led to sometimes cavalier prescribing and use practices, which culminated in lurid popular accounts of physical and psychological dependence (Gordon, 1979). Such an outcome, which reverberates to the present day in restrictive laws and litigious former patients, likely could have been avoided if physicians and patients had been made fully aware of the possibility of a withdrawal syndrome upon discontinuation and the liability of these compounds for abuse. In fact, limited data on physical dependence with the first clinically available benzodiazepine, chlordiazepoxide (Librium) had been published (Hollister et al., 1961), but the implications had not been fully pursued. Current attention to full characterization of candidate medications in the course of drug development is key to preventing such unfortunate episodes.

## 2. Behavioral methods for abuse liability evaluation in animals

The FDA Guidelines cited above set forth the definition of abuse liability that is most relevant for the purposes of the present paper: "The term 'abuse liability' refers to the likelihood that a drug with anabolic, psychoactive or central nervous system (CNS) effects will sustain patterns of non-medical self-administration (SA) that result in disruptive or undesirable consequences". (p. 3). The FDA document pointed out that there is no single test or assessment procedure likely to offer a complete characterization of abuse liability. Rather FDA assessment is based upon review

of all available data that concern the action of the substance. The Guidelines (p. 4) specifically request information on receptor binding, bioavailability, pharmacokinetics and metabolism, characterization of active metabolites, the likelihood and severity of undesirable consequences, subjective effects, reinforcing effects, discriminative effects, effect of abrupt abstinence after chronic administration, effects of supra-therapeutic doses, administration after destruction of the original dosage form, and, where available, epidemiological data on use, abuse, and adverse consequences.

Of the types of behavioral data the FDA requests, those on *reinforcing*, *discriminative*, and *physical-dependence-producing* effects are typically obtained most completely in laboratory animals, though they may be supplemented by data on one or more of these effects in humans (Griffiths et al., 2003, this volume). The present paper focuses on rationale and considerations for assessment of these three effects of a centrally acting compound; these data form the core of a behavioral assessment package, regardless of pharmacological class of the compound being studied.

Reinforcing effects are relevant to understanding whether the compound might maintain extra-therapeutic drug seeking and drug taking. Discriminative effects are relevant to understanding how similar the interoceptive stimulus effects of the compound are to other psychoactive compounds, which, for a compound in development, can include those currently marketed for the intended indication. Physical-dependence assessments provide data on the effects of abrupt drug withdrawal after chronic use, which are important both for determining whether a gradual drug cessation regimen should be designed for clinical use and for considering whether a withdrawal syndrome might contribute to extra-therapeutic drug use.

The purpose of the present paper is similar to that of the paper on abuse liability assessment in humans in this volume (Griffiths et al., 2003). That is, it describes methodologies that are currently applicable to assessments of centrally acting compounds in a context of general principles that are important for interpretation of data with respect to an FDA abuse liability evaluation. The rationale for methodological choices is supported by citation of the literature; but some rationale inevitably is in terms of our own experience, over more than two decades, with the development, use, and evaluation of procedures for comprehensive abuse liability assessments (Griffiths et al., 1979, 1985; Brady et al., 1987; Griffiths et al., 1992; Sannerud et al., 1992, 1993; Ator, 2000; Ator et al., 2000).

The present paper is not a history or critical evaluation of all methodologies that could inform a preclinical abuse liability assessment; that would require a monograph of its own. Reviews that discuss methodology for abuse liability evaluations in laboratory animals have

been published periodically over the past quarter century beginning with one by the [Committee on Problems of Drug Dependence \(1973\)](#). [Balster and Bigelow \(2003, this volume\)](#) review that literature, which shows both the evolution of thinking about methodologies that would be useful in abuse liability evaluation and the extensions of such assessments across pharmacological classes.

### 3. Cross-cutting methodological considerations

A primary intent of the present paper is to emphasize methodological considerations that must be made to be able to draw well-founded conclusions about a test drug's reinforcing, discriminative, and physical-dependence-producing effects. Regardless of the specifics of procedures used, certain overarching methodological considerations apply to any study that is intended to contribute to an abuse liability assessment.

#### 3.1. Positive and negative controls

Positive and negative control drugs should be used for comparisons. Positive controls should be compounds known to have abuse liability in the general population and, of course, to reliably show up as positive in the particular assay. Where possible, a positive control should be of the same pharmacological class and/or for the same intended clinical use, which makes comparison with the novel compound more meaningful. Of course, the placebo, or vehicle, will serve as a control for the drug administration procedures; but an active compound that can serve as a negative control is also useful. Negative controls are compounds that do have well-characterized behavioral effects at the doses studied but reliably show up as negative in the particular abuse liability assessment procedure. This confirms the ability of the assay to differentiate among behaviorally active compounds.

#### 3.2. Dose–effect evaluations

A value of an assessment in laboratory animals is the ability to characterize a full range of doses, including low doses that produce no effect in the assay compared to vehicle and high doses that clearly represent the upper limit of those that can be studied. Characterization of the effects of a full range of doses reveals the shape of the dose–effect function and, therein, the potency and efficacy of the compound. This can be particularly important for truly novel compounds. If the results for a particular assessment are negative in relation to the drug vehicle, the highest dose tested should be one that is unequivocally limited by, for instance, solubility or toxicity considerations.

#### 3.3. Multiple dependent measures

As implied in the listing of information taken into account by the FDA in its evaluation of an abuse liability profile, behavioral data other than those on reinforcement, drug discrimination, and physical dependence may usefully be supplied to characterize the likelihood and severity of undesirable consequences of drug taking. For example, drug effects on motor function, learning, memory, and appetitively motivated behavior may be evaluated. Assessment of multiple measures or of multiple dependent variables within an assay can provide the opportunity to detect drug effects that assure that a behaviorally effective dose range is being studied even if the effect for the behavior of primary focus (e.g. drug reinforcement) is negative. Multiple measures can also provide a basis for determining whether apparent tolerance to any particular effect of the drug occurs with repeated dosing.

#### 3.4. Pharmacokinetics

A plan to obtain blood samples that can be assayed for plasma or serum levels of the parent compound and active metabolites can be useful. Pharmacokinetic data are particularly important if results of a behavioral assay are negative even when the maximum possible doses have been studied. This outcome has not been common with psychoactive drugs in the past; but as more receptor-subtype selective compounds and/or partial agonists are developed, it may be a more common occurrence that even very high doses of a drug will not produce overt behavioral effects or decreases in response rates. Blood levels can assure confidence in adequacy of dosing and may, in some cases, provide a rational basis for selection of the high dose and temporal parameters of certain assays. The correlation between time course of behavioral effects and blood levels can be useful, particularly for determining contribution of clinically important metabolites to different behavioral effects.

#### 3.5. Choice of species

Continuity in physiology across mammalian species makes many species valid choices for preclinical studies. Drug reinforcement, drug discrimination, and/or physical dependence studies have been carried out and published most extensively for rats, dogs, and nonhuman primates (e.g. see citations in reviews in [Balster and Bigelow, 2003, this volume](#), and below). Nonhuman primates, particularly old-world monkeys (usually macaques and baboons), offer advantages as subjects at some phase of abuse liability assessments. This is because of the close phylogenetic link between human and nonhuman primates, and similarities in drug

metabolism and other physiological characteristics (Rosenblum and Coe, 1985; Friedman and Popova, 1988; Bennett et al., 1998). A practical consideration is that the longevity of nonhuman primates in the laboratory facilitates completion of dose–effect studies within subjects for the test compound and the comparison compounds. Nonhuman primates that are used in behavioral work tend to be conserved across studies rather than sacrificed at the end of a study. Not only is this cost-effective, but it can be time-saving in that animals do not need to be newly trained to begin study of a new drug, and permits cross-drug comparisons for the same subject under the same procedure. It is not uncommon for a laboratory to restudy drugs in such animals to determine if time or pharmacological history has altered the behavioral outcome.

### 3.6. *Experimental design*

#### 3.6.1. *Group designs*

Due to the time and expense of many behavioral training procedures, within—rather than between—group designs are common. That is, the same subjects are studied under all the vehicle and drug dose conditions. The same group may be used also for study of the positive and negative control drugs (i.e. both drug and dose are within-group variables), or additional groups are used for study of each drug (i.e. drug is a between-group variable, and dose is a within-group variable).

When animals are trained to perform specific behaviors to certain criterion levels, experimental manipulations typically are not made until stability on one or more dependent measures is observed. Even when a criterion for change is stated in terms of number of sessions of exposure, it tends to be based on the investigator's experience with the number of sessions needed for a meaningful effect to occur and/or for stability in performance to be obtained. Under such conditions, where random assignment is not the rule, group designs rely on nonparametric statistics for data analysis.

#### 3.6.2. *Single-subject designs*

An alternative to a group design is the single-subject design, which is commonly used in basic experimental analysis of behavior and in behavioral treatment research; a similar approach is used in study of basic physiological processes in a living organism (Sidman, 1960; Krishef, 1991; Bordens and Abbott, 1996). This design is particularly useful when the species is difficult or expensive to obtain and/or the conditions of study are labor-intensive and/or expensive. Use of the single-subject design in behavioral work is predicated on the assumption that if the independent variable is a strong determinant of behavior, then its effects should be capable of reliable demonstration in an individual

subject. Thus repeated study of each subject at each dose level is common. Replication of the effect in additional subjects determines its generality.

Good experimental control of conditions that may affect experimental outcome is particularly critical in single-subject designs; but unlike within-subject group designs, individual parametric manipulations may be necessary to obtain important commonalities across subjects in baseline performance or to demonstrate the experimental phenomenon. For example, in study of drug effects, the range of doses may need to differ across subjects to obtain a full characterization in every subject. This is more often true of nonhuman primates than rodents due to greater variations in age, weight, and, likely, metabolism among nonhuman primates in behavioral laboratories (i.e. inbred laboratory rats tend to be purchased in groups of the same age, but monkeys in a study may include ones that have been in the laboratory awhile as well as ones that are new).

Use of a single-subject design does not preclude use of statistical approaches in data analysis (Ator, 1999; Davison, 1999; Crosbie, 1999; Shull, 1999); but presentation of the data of the individual subjects is necessary for demonstration of the generality of effect. Group means can be shown also if they well-summarize the effect for the individual subjects. Convincing demonstration of an effect in each of three or four subjects has been accepted by those sophisticated in appropriate use of the single-subject design. If a subject does not exhibit the same effect as the others, even with manipulation of dose range, manipulation of drug pretreatment time or other such variable may result in congruence across animals. Otherwise study of one or more additional subjects can serve to characterize the probability of the phenomenon under those particular experimental conditions.

## 4. **Drug self-administration**

### 4.1. *Background*

A key component of preclinical abuse liability assessment is to determine whether the compound will be self-administered. The goal is to assess the ability of the drug to serve as a reinforcer, which is operationally defined as determining whether drug delivery maintains behavior at a meaningfully higher rate than does the control (usually vehicle delivery). The model originally was developed by use of intravenous drug delivery through chronically indwelling catheters in rats and monkeys (Weeks, 1962; Thompson and Schuster, 1964). A critically important finding was that the animals did not have to be made physically dependent on the drug before it would serve as a reinforcer. The intravenous model quickly came to be seen as valid for studying

variables related to human drug taking because, with few exceptions, drugs that were known to be abused by humans were shown to serve as reinforcers in animals; and drugs that were not abused by humans did not (Schuster and Thompson, 1969).

#### 4.2. *Route: intravenous and oral*

Drug reinforcement assessments in laboratory animals tend to use the intravenous route of administration, regardless of species and drug class (for rats: van Ree et al., 1978; Collins et al., 1984; Roberts et al., 1999; and monkeys: Slifer and Balster, 1983; Lukas et al., 1986; Woolverton et al., 1986; Griffiths et al., 1992; Weed et al., 1997; Ator, 2000). The i.v. route has the advantages that delivery of drug to the CNS is almost immediate. A disadvantage of the i.v. route is the technical challenge of maintaining viable long-term indwelling i.v. catheters, but this technology has been well-worked out (review in Meisch and Lemaire, 1993). Another disadvantage is that solubility characteristics of a test compound may unduly restrict the range of doses that can be examined in i.v. administration within reasonable parameters of drug delivery duration and volume.

Given that most newly developed drugs for clinical use are intended for oral administration, use of the intragastric or oral route for study of drug reinforcement has good rationale and face validity. Drug solubility limitations are more easily overcome than with i.v. delivery. Drug can be delivered in suspension and in larger volumes. Despite the refinement of use of intragastric catheters (Lukas et al., 1982) and their use for abuse liability evaluation (Yanagita, 1977), the reliability of the intragastric method across subjects and its validation for abuse liability evaluation across pharmacological classes has been questioned (Woolverton and Schuster, 1983 cf. Meisch and Lemaire, 1993).

Oral SA by mouth (per os, p.o.) has been more successfully established than has i.g. SA. The primary difficulty of oral SA for abuse liability assessment is overcoming taste as a factor that limits drug exposure and/or independently produces a negative result. Use of fluid restriction as an inducing variable is not generally useful because it confounds interpretation of drug reinforcement. Forced consumption of an adulterated solution seems to work against drug preference once water no longer is restricted, possibly due to pairing with dehydration (review by Myers and Veale, 1972). Mixing the drug with a palatable (e.g. sweetened) solution has been useful for promoting ingestion of behaviorally active doses; but the sweetener must be gradually reduced from the mixture, with drug consumption continuing, to be able to assess drug reinforcement per se. A conceptually similar methodology is to mix the novel drug with a drug already functioning as a

reinforcer and then fade out the original drug (review in Meisch and Lemaire, 1993). Difficulties with interpretation of the test results as demonstration of reinforcement by the novel drug when mixtures are used have been identified, however (Falk and Lau, 1993).

Procedures were developed in study of alcohol SA to habituate an animal to the taste while exposing them to gradually increasing concentrations, and these methods have proven useful in the study of p.o. intake of other drugs (Meisch, 1977; Turkkan et al., 1989; review in Meisch and Lemaire, 1993). However, the number of studies that have been relatively straightforward assessments of whether a compound would serve as a reinforcer via the oral route are far fewer than for i.v. SA (Carroll and Meisch, 1978; DeNoble et al., 1982; Ator and Griffiths, 1983c; Carroll and Stotz, 1984; Carroll et al., 1984; Ator and Griffiths, 1992; Falk et al., 1994 cf. Vivian et al., 1999). Once drinking is established, methods for manipulating dose need to be considered in relation to whether holding volume of delivery or drug concentration constant is the method by which dose–effect relationships are to be examined. Control of food intake is important to oral drug SA methodology. Food in the stomach will influence drug absorption, so access typically is restricted to periods of time after the experimental session. Furthermore food restriction has been shown to facilitate acquisition of oral SA, and its role in assessment of demonstrations of drug reinforcement needs to be considered (Carroll and Meisch, 1984).

#### 4.3. *Conditions of drug availability*

##### 4.3.1. *Timeouts*

Choice of parameters of drug availability also influence comparisons of relative efficacy of drugs as reinforcers. For example, i.v. pentobarbital initially seemed not to maintain drug taking as well as i.v. amphetamine, but when the influence of sedative effects of self-administered drug on subsequent responding are minimized, pentobarbital maintains SA as well as an abused stimulant (cf. Goldberg et al., 1971a; Griffiths et al., 1981). Thus imposition of timeouts after each injection reduces drug accumulation and facilitates assessment of the ability of any given dose to maintain self-injection. In our laboratory, we adopted a 3 h post-injection timeout, with experimental conditions in effect 24 h/day. While this enforced inter-dose interval is perhaps unnecessarily long for study of some compounds, it permits assessment of a broader dose range than might be safe or possible with shorter timeouts, and facilitates use of the same procedure for comparison of SA across a wide range of pharmacological classes and pharmacokinetic characteristics. Other labs use no timeout or a shorter one and conduct sessions of shorter durations (e.g. 2 or 3 h session durations). Of course,

with any length of timeout, toxicity of single high doses must be considered. With drugs that are in development, such information often has been collected prior to beginning an abuse liability investigation and may usefully inform dose range selection. Where such data are not available, preliminary work with delivery of increasing i.v. doses and/or pharmacokinetic data can help define the dose range.

#### 4.3.2. *Requirements for drug delivery*

A wide variety of well-characterized schedules of reinforcement are available and many have been used to study drug reinforcement (Johanson, 1978; Spealman and Goldberg, 1978; Young and Herling, 1986; Katz, 1989). A schedule of reinforcement is a rule that specifies the contingency between making a particular response (usually a lever press) and delivery of the putative reinforcer. Schedules that require a fixed number of lever presses per injection (a fixed ratio (FR) schedule) are common in SA research. With an FR schedule, the rate of SA within the experimental session varies, constrained primarily by the effects of cumulated drug and/or the duration of any timeout. The most common alternative is a fixed interval (FI) schedule, in which the first response after a fixed period of time produces the injection. Responses prior to that response have no scheduled effect, but the pattern and rate of those responses can vary as a function of the drug dose available. Combinations of FR and FI requirements in the form of second-order schedules have been favored for some purposes. For example, one can arrange brief visual or auditory feedback at the completion of groups of responses under an FR contingency, but deliver the drug under a long FI contingency, and then end the session after the injection. This arrangement has facilitated assessing rates of responding maintained by a drug independently of any direct behavioral effect of previous drug injection.

If a drug has been shown to serve as a reinforcer under one schedule of reinforcement, it typically can also be shown to serve as a reinforcer under another schedule of reinforcement, but some schedules of reinforcement may be more useful for maintaining long sequences of responding, while holding interdose interval constant. Fixed interval and second order schedules have been particularly favored in this regard. Thus rate of responding as a function of dose is the primary dependent variable. In studies that use FR schedules in a fixed duration session, and leave number of injections per session free to vary, response rate and rate of self-dosing (e.g. self-injection) are almost interchangeable as dependent variables.

#### 4.3.3. *Demonstration of reinforcement*

In i.v. SA studies, presentation of drug doses and vehicle in sequential conditions is usual. Concurrent

availability of vehicle and drug is technically more challenging. In oral SA, choice between drug and vehicle traditionally has been the more common procedure. Use of identical schedules of reinforcement on access to each fluid, however, has been found to make differentiation of drug reinforcement easier. Under such conditions, equal volumes of fluid might be consumed from both options when there is free access to both; but drug reinforcement emerges as the response requirement (e.g. FR value) for both fluids is raised (Meisch and Lemaire, 1993).

#### 4.4. *Substitution procedures*

A commonly used method for assessing SA of a novel compound via the i.v. route is to substitute a dose of the test drug or its vehicle for a dose of a reinforcing drug that is already maintaining SA at a reasonably high rate in a lab animal (Johanson and Balster, 1978). This 'drug substitution' procedure has important advantages over merely making the test drug available and waiting to see whether the animal learns to self-inject it (i.e. an acquisition procedure). In a substitution procedure, self-injection of a test dose is highly likely shortly after it is made available, thus assuring contact with the test compound relatively quickly. When the baseline compound replaces the test condition, return to the criterion level of self-injection typically is rapid, which confirms the patency of the catheter in case self-injection of the test dose had resulted in low or zero rates of self-injection. Then, once baseline performance is at a criterion level, another test dose or drug vehicle can be made available which enables determination of the dose–effect relationship fairly efficiently.

Critical elements of a substitution procedure are choice of the baseline drug and assessment of a sufficiently long period of substitution to conclude that transition from the baseline drug condition has occurred. That the period of substitution is sufficiently long is based on inspection of the rates of responding and/or self-injection once the test dose is available. These may go up, down, or stay the same relative to baseline; but, depending upon the parameters of the conditions of availability, the rate and pattern of responding becomes clear after some number of self-injections. The length of the period of substitution of each test dose should be according to a well-considered criterion in relation to the conditions of availability and the dependent variable(s) and is best standardized across drugs to facilitate cross-drug comparisons. For example, rate of responding or rate of self-injection may have to meet a stated stability criterion before return to the baseline condition; or the same period of substitution could be used for study of each dose; or some combination of these criteria could be used.

The baseline drug condition should be one that maintains moderate to high stable rates of self-injection and one that does not produce physical dependence, which could complicate assessment of the reinforcing effects of the test drug. It is not that a baseline drug should be one that never produces physical dependence, just that there should not be observable withdrawal effects when it is removed under the conditions in which it is serving as the baseline drug. The pharmacokinetics of the baseline drug should be such that the parent compound and any active metabolites are fairly quickly eliminated after injection. Codeine, cocaine, pentobarbital, and methohexital have been favored as baseline drugs in i.v. abuse liability evaluations (Johanson and Balster, 1978; Griffiths et al., 1991; Johanson, 1987; Gerak et al., 2001). Where drugs can be compared, those that maintain SA under substitution procedures also generally maintain SA in different species, in drug naive animals, and when the drug is provided following a period of vehicle availability (Pickens et al., 1981; Collins et al., 1984; Griffiths et al., 1991; Spear et al., 1991).

Substitution procedures for p.o. drug SA have been used when phencyclidine, pentobarbital, or alcohol was the baseline drug (Carroll and Stotz, 1984; Ator and Griffiths, 1983c; Carroll et al., 1984). Although alcohol maintains behavior very well, methohexital did not maintain SA after its substitution for alcohol. In contrast, methohexital did maintain SA after substitution for pentobarbital. We speculate that the reason may have to do with the qualitative difference in taste between alcohol and other drugs; habituation to a bitter taste permits oral dosing with a range of drugs in baboons but not with alcohol (Turkkan et al., 1989). When taste habituation procedures were used in baboons that had failed to consume methohexital that was substituted for alcohol, drug reinforcement was well-demonstrated.

#### 4.5. *Effect of baseline or drug history*

In our studies, the baboons typically range from those with little previous self-injection experience to those with a great deal of experience, albeit the histories of no two animals will be the same. We have not found differences in either rate of self-injection or reinforcing efficacy of a compound per se based on pharmacological history per se. In fact, indication of such an effect would be extremely interesting and worthy of systematic study.

Another way in which ‘history’ can be defined, however, is in terms of the pharmacological baseline in substitution procedures, that is, the immediate history, or pharmacological context, in which a novel drug is studied. Phencyclidine, dexodrol, and dextrorphan were self-administered by monkeys when substituted for ketamine for only a single session but not when

similarly substituted for codeine (Young and Woods, 1981). Bergman and Johanson (1985) found that diazepam was demonstrated to be reinforcing in monkeys when substituted for 5–14 sessions for pentobarbital but not cocaine. Likewise Beardsley et al. (1990) found that the NMDA antagonist MK801 (dizocilpine) was self-administered when substituted for a few sessions off a PCP but not a cocaine baseline. Interestingly oral methohexital reinforcement was demonstrated more reliably when it was substituted for pentobarbital than for PCP (Carroll et al., 1984). These data indicate that immediate drug history, or context of drug substitution, can influence conclusions about the reinforcing efficacy of test drugs when the assessment is made close to the beginning of the period of substitution (see also Schlichting et al., 1970). This interpretation is bolstered by the fact that other studies have showed that drug reinforcement was demonstrated when a test drug was substituted. For a longer period of time off the baseline that did not yield a positive result in the studies cited above (Griffiths et al., 1981, for diazepam substitution off a cocaine baseline; cf. Grant and Johanson, 1987). The message of these studies taken together is that SA behavior in the period shortly after substitution for a baseline drug is more likely to be influenced by immediate history than SA behavior in a later period. These data thus support choice of a stability criterion or rationally selected length of study when substitution procedures are used.

#### 4.6. *Relation of drug reinforcement in animals to measures in humans*

The positive correlation between those drugs that have been abused by humans and those drugs that animals self-administer is clear for opioids, stimulants, sedatives, dissociative anesthetics, and others (Table 1 in Griffiths et al., 1980). Griffiths and Balster (1979) summarized data that showed the good correspondence between opioids that were self-administered by monkeys and those that produced morphine-like signs, symptoms, and subjective-effects in humans. One pharmacological class that, to date, appears as an exception to this correspondence is that of serotonergic hallucinogens, notably lysergic acid diethylamide (LSD, Johanson and Balster, 1978; Griffiths et al., 1980), which are abused by humans but not self-administered by animals. It should be pointed out, however, that the finding that animals generally do not self-administer serotonergic hallucinogens is consistent with the fact that most people who try hallucinogens do not continue to use them, and those that use them usually do so at an extremely low rate.

When we compared results of i.v. SA studies of 12 sedative, anxiolytic, or muscle relaxant compounds in baboons (Griffiths et al., 1981; Griffiths et al., 1991) with subjective-effect measures for the same drugs from

studies in humans with histories of sedative abuse (Preston et al., 1989; Sannerud et al., 1991b cf. Griffiths et al., 2003, this volume), we found a positive relationship of baboon SA results with the human drug liking measures for all except methocarbamol (Table 1). Methocarbamol was not self-administered by baboons, but it was ‘liked’ by humans. In this instance, baboon SA methods appear better than human methods at predicting actual abuse, because rate of abuse of methocarbamol is very low.

Conversely, discrepancies between animal SA results and drug abuse by humans have been noted in the fact that animals self-administer some drugs that have not proved to be abuse problems in humans and may not show up as positive in human laboratory studies (Woods, 1983; Rush et al., 2001). In some cases, the differences in results between laboratory studies in humans and animals may be due to differences in the effective dose range, route, or other variables (see discussion in Rush et al., 2001, for bupropion). However, drug availability, form of distribution, and socio-cultural variables influence actual patterns of drug abuse in any given time period. Thus a positive finding in an animal SA study may be interpreted as indicating that abuse is likely to arise under conditions of wide availability and low levels of legal control.

#### 4.7. Relative reinforcing efficacy

One question often raised about the drug SA methodology is whether drugs can be differentiated with

Table 1  
Results of ‘liking’ assessments in humans with histories of drug abuse and assessments of drug reinforcement in SA studies in baboons for sedative/anxiolytic drugs

Drug	Liking	SA
Pentobarbital	+	+
Alprazolam	+	+
Diazepam	+	+
Flunitrazepam	+	+
Lorazepam	+	+
Triazolam	+	+
Zaleplon	+	+
Zolpidem	+	+
Abecarnil	0	0
Bretazenil	+	+
Buspirone	0	0
Tandospirone	0	0
Methocarbamol	+	0

Note: The ‘+’ sign indicates the drug was liked or SA greater than placebo. The ‘0’ indicates the drug was not liked or not SA greater than placebo. For references for human studies, see Griffiths et al. (2003, this volume); baboon studies: Griffiths et al. (1981); Griffiths et al. (1991); Griffiths et al. (1992), Sannerud et al. (1991b, 1992, 1993) and Ator (2000), and unpublished results for bretazenil and flunitrazepam.

respect to their relative ability to maintain SA. Even when full dose–effect curves are compared, evaluation of the relative reinforcing efficacy of a particular drug compared to others is subject to numerous caveats. Some drugs that do not maintain SA very well under one set of contingencies, will do so more efficaciously under others. The example above about pentobarbital reinforcement compared with that of amphetamine is one case in point. Studies of i.v. nicotine reinforcement in laboratory animals is another (Ator and Griffiths, 1983b; Spealman and Goldberg, 1982). As a third, delta 9-tetrahydrocannabinol now has been shown to maintain self-injection in monkeys whereas previously this compound was considered one of the exceptions to the good correspondence between those drugs abused by humans and self-administered by laboratory animals (Tanda et al., 2000 cf. Griffiths et al., 1980).

One definition of a basis for differentiating relative reinforcing efficacy has incorporated the qualifier that one drug may be considered more reinforcing than another if it maintains SA under a wider range of conditions of availability, across laboratories, and species (Schuster and Thompson, 1969; Griffiths et al., 1979; Johanson et al., 1987). By this criterion, cocaine is an excellent example of a compound with high relative reinforcing efficacy. That is, it is very effective in training species of animals to perform responses to produce it, and it maintains responding under many different conditions, including those with high response requirements (Johanson and Fischman, 1989). Another CNS stimulant compound, nicotine, has not proven as efficacious in maintaining behavior in the laboratory (Di Chiara, 2000).

The progressive ratio (PR) schedule of reinforcement has been used more frequently in recent years to determine whether rank orderings can be made among drugs based on the highest response requirement that a compound will sustain (i.e. the response requirement that serves as the ‘breaking point’; reviews in Richardson and Roberts, 1996; Stafford et al., 1998). Under a PR schedule, the response requirement for drug delivery increases according to some predetermined progression within or across experimental sessions. While these efforts are important in their own right, they are most likely to be useful for within-class rankings (Griffiths et al., 1979; Weed et al., 1997; Roberts et al., 1999), but even then methodological pitfalls must be resolved. These include, but are not limited to, equating experience with the response-incrementing procedure itself across subjects, choosing an appropriate ‘breaktime duration’ given the duration of effects of all the drugs to be compared, and taking account of the effect of drug accumulation on response rates. Comparison of maximum breaking points should be based on full dose–effect determinations for each drug carried out under the same procedures.

Another possible methodology for comparing drug reinforcing efficacy is a choice procedure. This procedure has been fruitfully applied to determine whether the typical inverted U-shaped dose–effect function seen in studies of i.v. drug SA can be interpreted as indicating that the effects of high drug doses are aversive. Johanson and Schuster (1975) systematically examined choice between i.v. saline and doses of cocaine and also choice between lower and higher doses of cocaine in monkeys. They found that all doses of cocaine were chosen over saline on more than 75% of the opportunities; and the higher of two doses of cocaine was chosen on more than 50% of the opportunities. Convincing evidence that orally delivered pentobarbital was serving as a reinforcer compared to its vehicle was provided in choice procedures when the response requirement for obtaining either substance was increased substantially; and the same approach was used to demonstrate that higher concentrations of oral pentobarbital would maintain more behavior than lower ones (Lemaire and Meisch, 1984; Meisch and Lemaire, 1988). These findings point up a potential pitfall in using choice procedures to compare relative reinforcing effectiveness of two drugs. That is, procedures have to be designed that eliminate or control for the possibility that a particular choice is based on the quantitative dimension of dose than on the putative qualitative dimension of one drug's reinforcing efficacy compared to another. For example, given that both Drug X and Y were shown to be reinforcing compared to their vehicles, a strong indication of greater reinforcing efficacy for Drug X would be if a given dose of Drug X were preferred over all doses of Drug Y.

An approach to examining relative reinforcing efficacy that may be very useful for comparing drugs within and across classes is to employ the concepts and methods of behavioral economics. These principles, first extensively worked out by Hursh and his colleagues for food-maintained behavior in laboratory animals, have been extended to assessments of drug taking in both humans and animals (Hursh, 1980; Winger et al., 1996). Measures of cost (e.g. responses required) in relation to amounts of a commodity (e.g. dose of a drug) are used to construct demand curves (e.g. drug obtained as function of response requirement). Demand curves can be compared across commodities or in the context of other manipulations to determine demand's relative sensitivity to change. Demand curves and related analyses (Meisch, 2000; Rowlett, 2000) are being used both to compare reinforcing efficacy of compounds and to assess whether a potential drug abuse treatment medication might increase sensitivity to response cost, which in turn could produce a decrease in drug-seeking behavior.

## 5. Drug discrimination

### 5.1. Background

In drug discrimination procedures, animals are trained to respond differentially depending on the nature of the drug pretreatment. The goal is to train the animal to use the drug effect as a cue (technically termed a discriminative stimulus) as to which response to make to obtain some commodity (or, in some procedures, to avoid an aversive stimulus). The drug discrimination procedure has been characterized as providing information analogous to a human testing situation in which people who are experienced in illicit use of psychoactive drugs categorize test drugs as similar or dissimilar to drugs with which they are familiar (Griffiths et al., 2003, this volume). Although such pharmacological categorization by people sometimes is pharmacologically imprecise (e.g. some classified alprazolam as an opiate, Evans et al., 1994), categorization by animals is more pharmacologically precise, because, in the usual, two-choice, assay, the animal is explicitly trained only to differentiate one dose of one drug from a non-drug control condition. Because a drug discrimination is based on a drug's interoceptive stimulus effects, determining whether a new psychoactive drug shares that effect with one currently in clinical use and/or subject to abuse is a critical part of a new compound's pharmacological profile. As will be discussed below, these data provide a different dimension of a drug's effects than do SA data.

### 5.2. Training and testing procedures

The most frequently used procedure involves a two-lever choice situation in which the animal must learn that operation of one lever (e.g. the left one) will produce a food pellet if the animal is pretreated with the training drug but operation of the other one will not. The animal also learns that operation of the other lever (e.g. the right lever) produces food if the training drug effect is not present. Usually a vehicle is administered before those sessions. After a period of daily training in which the two types of sessions alternate, quite reliable discrimination performance is generated. A wide variety of psychoactive drugs have been used as training drugs (Overton, 1984). Whether the training drug would serve as a reinforcer is not an issue (i.e. animals have been trained to discriminate compounds that are not self-administered, such as buspirone, Ator, 1991).

Once the animal's performance reaches a preestablished criterion level, test sessions are conducted in which novel drug conditions are presented. When novel doses of the training drug are administered, well-trained animals respond on the drug-paired lever, except that after very low doses, they respond on the 'not drug'

lever. Under certain training and testing procedures, animals will respond on both levers at intermediate doses of the training drug, thus permitting determination of a 'threshold' dose (Ator, 1990 cf. McMillan and Li, 2000). The primary measure regarding test session performance is either percentage of training-drug-appropriate responding or percentage of animals that predominantly made that response. A second valuable piece of data is response rate. Rate not only serves as a separate piece of information about the drug's effects, a significant change in response rate confirms that a behaviorally active dose range was tested. This is important if the discriminative effects of the drug are totally unlike the training drug condition (i.e. if all responding is on the 'not drug' lever).

### 5.3. *What determines test results?*

Research that directly tested 'what is trained' (Overton et al., 1983) has confirmed that the trained discrimination is not drug versus placebo, but 'training drug' versus 'not training drug.' Drugs from the same or similar pharmacological classes tend to dose-dependently occasion responding on the lever that has been paired with the training drug, while those from different classes do not (Overton, 1984; Järbe, 1987). Furthermore the range of drugs to which an animal generalizes (i.e. responds on the training drug lever) appears primarily determined by the overlap in the neuropharmacology of the training and test drugs. Global effects, such as ability to induce somnolence or euphoria, do not appear to play a role in determining the outcome of a test. For example, baboons trained to discriminate the benzodiazepine agonist lorazepam did not generalize to either morphine or meprobamate and only partially generalized to barbiturates (Ator and Griffiths, 1989a,b, 1997). Impressive specificity can be achieved within a receptor system. By judicious choice of training drug, a benzodiazepine receptor agonist can be differentiated from barbiturates (Ator and Griffiths, 1989a,b, 1997), opioid mu agonists can be differentiated from kappa agonists (Woods et al., 1988), and a 5-HT<sub>1A</sub> agonist can be differentiated from a 5-HT<sub>1B</sub> or a 5-HT<sub>2</sub> agonist (Glennon, 1986).

Choice of training drug(s) should be made on the basis of what is known about mechanism(s) of action of the compound(s) to be tested. Testing a novel compound in multiple relevant training conditions may be particularly informative. For example, the partial benzodiazepine agonist activity of panadiplon (U-78875) was best shown by comparing its effects in a diazepam training condition (which appears selective for drugs that potentiate gamma-aminobutyric acid, GABA) with those in a lorazepam training condition (which appears selective for full agonists at the benzodiazepine site on the GABA<sub>A</sub> receptor; Ator and Griffiths, 1999). If a

drug has actions in more than one neurotransmitter system, a drug discrimination analysis can help determine whether activity in one system plays a bigger role in its interoceptive stimulus effects than the other (e.g. buspirone, Ator, 1991).

Use of the novel compound itself as a training drug can be fruitful. Failure to use the novel drug as a training condition can lead to an incomplete understanding because of the well-documented phenomenon of asymmetrical cross-generalization. For example, animals trained to discriminate pentobarbital generalized to lorazepam, but, as mentioned above, animals trained to discriminate lorazepam have not reliably generalized to pentobarbital (Ator and Griffiths, 1983a, 1989a,b, 1997). Asymmetrical cross-generalization can point to which of multiple possible mechanisms is necessary for producing a particular trained effect. For example, the trained lorazepam stimulus appears to be highly dependent on full agonist activity at the benzodiazepine site on GABA<sub>A</sub> receptors (Ator and Griffiths, 1999).

Training drug dose can influence test outcome under some conditions. Dose–effect curves for test drugs that dose-dependently occasion the training drug response will be shifted to the right as training drug dose increases (Holtzman, 1983; Shannon and Herling, 1983; Ator and Griffiths, 1989b). Some qualitative changes in generalization can occur as well. For example, the mixed-action opioid nalbuphine fully shared discriminative effects with a lower but not a higher training dose of morphine (Shannon and Holtzman, 1979).

A valuable component of a drug discrimination assay is the ability to use a specific receptor antagonist, if one exists, to study mediation of the discriminative effects of the test compound. If generalization is obtained, then interactions with the antagonist can be studied to determine whether this affect depends on binding at this site.

### 5.4. *Role of pharmacological history*

The spectrum of drugs to which an animal generalizes can be broadened by successively adding additional drugs as training drugs paired with the same response (Overton et al., 1983; McMillan et al., 1996). On the other hand, experience with multiple drugs as test drugs has not appeared to influence the probability of generalization (but cf. Barrett and Olmstead, 1989). In our own experience with baboons and rats trained to discriminate various benzodiazepine agonists or pentobarbital, we have retested compounds in the same animals at later dates or explicitly counterbalanced order of testing novel compounds, and failed to find any effect of testing experience on the qualitative or quantitative characteristics of the test results. This is not to say that this could not occur under some training conditions, but to date

testing experience has not appeared to be a confound in drug discrimination research.

Prolonged experience with the training drug does not result in tolerance to its discriminative effects. Tolerance to any initial effects on response rate is common, but the very training procedure itself prevents loss of stimulus control as a function of chronic drug. In fact, tolerance to discriminative effects must be demonstrated by testing after chronic dosing over a period in which training is suspended (Young and Sannerud, 1989).

### 5.5. Interpretation of drug discrimination data

Several reviews have shown that SA of drugs from a pharmacological class covaried with whether or not those drugs shared discriminative stimulus effects with a recognized drug of abuse in the same class (Schuster et al., 1981; Woods et al., 1982; Balster and Willetts, 1988). At the same time, however, it is recognized that this is not a necessary relationship, especially given that one has no way of knowing or assuring that the effect that subserves a trained discrimination is the same effect that maintains SA. For example, both hexobarbital and phenobarbital shared discriminative effects with pentobarbital but were not self-administered (Herling et al., 1980; Ator and Griffiths, 1989a; Griffiths et al., 1991). Ator (2002) directly tested, in individual subjects, whether the relation between discriminative stimulus and reinforcing effects was invariably positive for five GABAergic drugs and found that it was not. Thus in testing a novel compound, a failure to demonstrate cross generalization with an abused, pharmacologically dissimilar compound does not have any bearing on abuse liability of the novel drug. Conversely the finding that a compound does share discriminative effects with an abused drug does not predict that the compound also will be reinforcing in an SA paradigm (Ator, 2002).

Given the dissociation that can occur between a drug's discriminative and reinforcing effects, an intriguing possibility arises. That is, discriminative effects may be useful for predicting patient acceptability of a psychoactive compound. For example, benzodiazepines have good patient acceptance when long-term treatment is required, but they also are subject to abuse and produce physical dependence. A candidate drug for replacing benzodiazepines for long-term treatment was buspirone, but patients with a history of benzodiazepine treatment do not respond well when switched to buspirone (Goa and Ward, 1986; Wang et al., 1990). If a novel drug were to share discriminative effects with a clinically useful benzodiazepine but not be self-administered, it could indicate a good probability of patient acceptance with lower abuse liability than with the benzodiazepine. A similar outcome for a dopaminergic drug might facilitate treatment of cocaine abuse.

## 6. Physical dependence

### 6.1. Background and overview

Physical dependence is manifested as time-limited biochemical, physiological, and behavioral disruptions upon termination of a regimen of chronic drug administration. When any chronically administered drug no longer is given, some sort of 'discontinuation' or 'withdrawal' syndrome is not uncommon; this applies to peripherally as well as centrally acting drugs. The phenomenon often has been called an 'abstinence' syndrome in the case of psychoactive drugs in humans. The basis for any such syndrome is believed to be that a drug-induced adaptive syndrome occurs during chronic drug administration as part of a homeostatic process (Haefely, 1986; Meyer and Berger, 1998). Physical dependence thus is seen to be a logical result of adaptation to drug by the body; cessation of drug delivery necessarily calls for re-adaptation. A withdrawal syndrome should not be confused with mere absence of the effect the drug was producing (e.g. reduction of pain, anxiety, or depression).

The nature, severity, and duration of a withdrawal syndrome are determined by pharmacological and pharmacokinetic variables as well as by the nature of the physical systems that have adapted in the course of chronic drug administration. Different classes of compounds tend to have characteristic effects when drug taking is abruptly terminated (Jaffe, 1990; Meyer and Berger, 1998). The withdrawal syndrome can be dramatic and extremely unpleasant and have elements that extend over a protracted period (Meyer and Berger, 1998). While many withdrawal symptoms are not life-threatening, seizures, which characterize abrupt withdrawal from chronic barbiturates, can result in death if the individual is not monitored (Yanagita and Takahashi, 1970; Nishino et al., 1998). Assessment of abrupt drug withdrawal is important also because, with some drugs of abuse, most notably opioids, aversive effects of a withdrawal syndrome appear to play a major role in maintaining continued SA, even in the context of an expressed desire to stop taking the drug; laboratory animal data support this notion (Goldberg et al., 1971b,c).

In laboratory animals, the conditions that produce physical dependence and the nature of withdrawal syndromes have been most extensively characterized across species for opioids and sedatives, including alcohol (Kalant et al., 1971; Katz and Valentino, 1986; Woods et al., 1987, 1992; Carroll et al., 1990). Assessment of the effects of abrupt termination of chronic drug administration traditionally has been a critical component of the abuse liability assessment required by FDA for opioids and sedatives. One could argue, however, that study of the effects of abrupt cessation

of chronic drug administration is relevant to characterization of any psychoactive drug intended for clinical use and certainly is relevant to the evaluation of any truly novel compound. With any drug, it is important to determine whether a discontinuation syndrome will have significant consequences to health and safety. What has been lacking for a broader preclinical approach to assessment of drug withdrawal has been basic research on the effects of abrupt discontinuation of administration of pharmacologically diverse compounds that could serve as relevant standards for particular mechanisms of action. The following discussion is based on what has been generally true for studies of physical dependence with opioids and sedatives, and may serve as a useful orientation for preclinical work with other drug classes as well.

### 6.2. General considerations for chronic dosing

Probability of physical dependence increases as a function of dose and duration of treatment when drug is given chronically (e.g. daily or multiple times a day). With some drugs that can produce physical dependence, it may take several months of taking clinically appropriate doses for a person to have a discernible withdrawal syndrome (e.g. such as with the benzodiazepine anxiolytic chlordiazepoxide). Study of dose–effect relationships for producing physical dependence is the ideal, but a physical dependence assessment of even one dose can be particularly time-consuming and potentially expensive. Thus particularly for the purposes of an abuse liability assessment, a useful strategy is to choose a maximally stringent (i.e. aggressive) dosing regimen to answer the basic question of whether or not physical dependence is produced. If there is evidence of physical dependence when drug is abruptly withdrawn, then the qualitative nature of the withdrawal syndrome can be characterized. Parametric evaluations can be designed if further characterization of the conditions under which physical dependence is produced is desired.

For a maximally stringent dosing regimen, it is the possibility of negative results that should drive the choice of parameters. Failure to have used experimental parameters that can be seen as having pushed the limits of dosing for a physical dependence assessment can reduce confidence in interpreting a negative result. A common strategy in animal studies is to administer a very high dose of the test compound for a moderate duration. For example, a dose that is multiple times a behaviorally active dose, given by the same route, in the same species, could be given for a month.

Behavioral effects that traditionally have been used to determine when a ‘high’ dose has been reached (e.g. suppression of response rate in drug discrimination) may not be able to be used with novel compounds that do not produce the full spectrum of effects as the

standards. If the test compound has not produced behavioral effects in other assays in the same species, blood levels may be crucial to picking a dose to assess physical dependence potential. By the same reasoning outlined above for choosing dose per se, supra-therapeutic blood levels should be attained by the dosing regimen if blood level is what is used to select the dose for a physical dependence assessment.

Ideally assessment of physical dependence potential will use the route of administration that is planned for clinical use, which most often is oral. Intra-gastric delivery via a chronically indwelling catheter can substitute for drinking to assure reliable dosing. Computer control of pump systems can be used to assure dosing at programmed intervals. If intramuscular or intraperitoneal injection is preferred, the study likely will be more labor intensive.

With respect to frequency of dosing, the strongest test may be one in which the target receptor(s) can be presumed to be fully occupied continuously for the dosing period. This could be accomplished either by multiple drug deliveries, with intervals based on the pharmacokinetics of the drug, or by continuous infusion of the drug. In our laboratory, we characterized the barbiturate- and benzodiazepine-type withdrawal syndrome in the baboon by using continuous intra-gastric infusion to deliver a high total dose over 24 h for about a month (Lukas and Griffiths, 1982, 1984; Lamb and Griffiths, 1984, 1987, 1993; Sannerud et al., 1991a). Against that data base, once-per-day dosing conditions also have been assessed (Weerts et al., 1998; Ator et al., 2000).

### 6.3. Dependent measures

Behavioral assessments should be comprehensive and include normal and abnormal behaviors. Being able to observe changes in trained and untrained behaviors amplifies the scope of the assessment. For example, in our laboratory, we conduct structured observational sessions, but also assess operation of a lever that produces the daily supply of nutritionally balanced diet (i.e. an FR 10 schedule of reinforcement with 1 g food pellets in the baboon). We also assess fine motor coordination (Weerts et al., 1998; Ator et al., 2000). While the most severe withdrawal syndrome from particular drugs (e.g. morphine, pentobarbital) would be unmistakable, having an adequately long and extensively documented control condition makes assessment of less dramatic withdrawal syndromes possible.

Behavioral assessments during the dosing condition itself document the effects of the drug and any changes in effect across the course of the dosing. The withdrawal syndrome then can be characterized in relation to both the non-drug control condition and the chronic drug condition.

#### 6.4. *Withdrawal assessments*

When administration of a drug is abruptly stopped, the emergence of a 'spontaneous' withdrawal syndrome is of interest. This is the condition most similar to that under which a withdrawal syndrome might occur in a human. Although some signs might appear within the first 24 h, pharmacokinetic characteristics of the drug and any behaviorally active metabolites influence the time over which full expression of a withdrawal syndrome will occur; individual differences in elimination of the agonist also will influence time of onset, duration, and perhaps expression of withdrawal signs when drug administration stops (Okamoto and Hinman, 1983). Experimental assessment of the spontaneous withdrawal syndrome should extend long enough to determine whether changes observed are time-limited, that is, whether the behavioral profile reverts to that of the predrug baseline or appears permanently altered. If the latter is the case, the changed behavior may just represent a shift in frequency due to variables other than physical dependence. For example, food-maintained behavior in baboons took a month or more to return to control levels after chronic triazolam although other withdrawal signs ended after about 2 weeks (Ator et al., 2000); thus decrease in food-maintained behavior after drug withdrawal could be included as a feature of the withdrawal syndrome.

##### 6.4.1. *Precipitated withdrawal*

If a specific antagonist for the receptor targeted by the test compound exists, study of a precipitated withdrawal syndrome can be useful for assessing development of a physical dependence process. Use of naloxone or naltrexone with opioids is well-characterized (Katz and Valentino, 1986). Flumazenil and CGS 8216 are antagonists that have been useful in studies of physical dependence on ligands for the benzodiazepine binding site (Woods et al., 1987, 1992; Martin et al., 1995; Weerts et al., 1998; Ator et al., 2000). Given that doses of the agonist will be quite high, it is important that the antagonist dose is high enough to reverse completely the effects of that dose of the agonist (due presumably to complete displacement of the agonist from the binding site). For example, if the test compound has been shown to produce discriminative effects in another phase of the evaluation in the same species, one can determine whether the antagonist dose planned for the physical dependence phase will block discriminative effects of the same dose.

The withdrawal syndrome precipitated by antagonist administration typically has a quick onset, a brief duration, and may be more intense than that observed after drug withdrawal. There may be more reliability in the constellation of signs observed across all animals in precipitated than spontaneous withdrawal. This could

be an artifact in that animals typically are observed continuously in precipitated withdrawal (i.e. at least as long as the antagonist effects last); but they may be observed more intermittently in spontaneous withdrawal. On the other hand, there may indeed be a greater probability of idiosyncratic sets of withdrawal signs in spontaneous than precipitated withdrawal due to the metabolic processes involved in spontaneous withdrawal rather than the rapid displacement of agonist at the receptor in precipitated withdrawal.

##### 6.5. *Cross-dependence procedure*

Studies other than those of direct dependence can assess information relevant to the physical dependence potential of a compound. Cross-dependence testing is common with opioids, has been used with sedatives, and is a fairly efficient method of determining whether a novel compound can fully suppress signs of withdrawal from a standard compound (Yanagita, 1981; Katz and Valentino, 1986; Yutzenka et al., 1989; Lamb and Griffiths, 1993). In the cross-dependence procedure, an animal is made physically dependent on a standard compound of the pharmacological class of interest. Physical dependence is assessed by substituting vehicle for the drug for an appropriate period of time to be able to observe a withdrawal syndrome. Then the effects of administering a novel compound instead of the standard compound can be assessed for its ability to suppress the expected signs of withdrawal from the standard. If the test compound does prevent signs of withdrawal from the standard, it is commonly surmised that the test compound itself likely would produce physical dependence after chronic administration.

Cross-dependence testing is an efficient and useful procedure, but a disadvantage is that one cannot determine whether the nature of the withdrawal syndrome from the novel compound would be less severe than that from the standard. If it were to be, that in and of itself, could represent a benefit of treatment with the novel compound. Also partial suppression of withdrawal signs across a dose range of the test compound is more problematic to interpret and may suggest the need to study directly the ability of the compound to produce physical dependence.

##### 6.6. *Experimental history*

A history of opiate physical dependence and antagonist administration may result in sensitivity to an opiate antagonist long after the opioid physical dependence has ended (Katz and Valentino, 1986). Frequent administration of an opiate antagonist in the context of the development of opioid agonist dependence may attenuate the severity of withdrawal (Krystal et al., 1989 cf. Aceto et al., 1977).

Severity of flumazenil-precipitated withdrawal after a few days of diazepam appeared correlated with history of such experience (Lukas and Griffiths, 1984), but this effect did not occur if flumazenil challenges occurred after more than a week of administration of the benzodiazepine agonist (Lamb and Griffiths, 1985; Gallager et al., 1986; Ator and Griffiths, 1992; Martin et al., 1995; Ator et al., 2000). Although we have not arranged experimental conditions to study history, we have not found a difference among baboons in whether or not a withdrawal syndrome occurred after chronic administration of a benzodiazepine agonist as a function of previous drug or physical dependence history in our 20 years of dependence assessments in baboons.

## 7. Final comments

Obtaining profiles of the reinforcing, discriminative, and physical-dependence-producing effects of newly developed, centrally acting compounds in laboratory animals is essential for regulatory compliance and can be particularly useful in the drug development process. Drug SA, drug discrimination, and physical dependence assessment procedures each uniquely supply information that forms the core of an abuse liability assessment, but no one of them is definitive as to the level of legal control warranted under the CSA. Numerous factors determine such scheduling. For example, buprenorphine originally was placed in Schedule V of the CSA; yet it serves as a reinforcer in SA studies (Lukas et al., 1986). When buprenorphine was approved for outpatient prescriptions in treatment of opiate dependence in 2002, thus becoming more widely available, it was rescheduled to a higher level of control under the CSA, Schedule III.

The ultimate rationale for a comprehensive abuse liability evaluation, in both humans and animals, is first and foremost the need to provide the most accurate guidance to physicians and their patients on appropriate use of any centrally acting drug. Full understanding of the liability for abuse or the potential for a withdrawal syndrome upon abrupt cessation of use is essential to avoid unwarranted expectations that a new drug is a panacea (Cavallero et al., 1993; Watsky, 1996; Bottlender et al., 1997; Aragona, 2000 cf. Weerts et al., 1998). Actual abuse of a clinically useful compound by those prone to such misuse will be determined by variables that interact with the compound itself, such as the form in which the compound is made available. Abuse liability evaluations with compounds having novel mechanisms of action also will help to determine whether mechanisms that support drug reinforcement or physical dependence can indeed be separated from those that support the clinical usefulness of these newer

compounds. In such instances, abuse liability data have added scientific value.

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