The articles in this issue provide an excellent overview of the breadth of research relevant to drug addiction and the key role that preclinical research has played in advancing understanding of the processes that underlie substance use and dependence disorders, as they are coded and classified in the Diagnostic and Statistical Manual of Mental Disorders (4th ed., DSM-IV; APA 1994). The animal care and use issues in such studies, with few exceptions, are not unique to substance abuse research, which has much in common with studies in behavioral psychology, pharmacology, and neuroscience and indeed, as the articles in this issue illustrate, both draws from and contributes to the knowledge base in all three areas.

In this essay we review the importance of research, and of animal models in particular, to shed light on drug addiction. We then discuss issues that are unique to the institutional animal care and use committee (IACUC) review of protocols in the area of drug addiction research. These issues concern the types of drugs studied, their legal controls, and sources; psychoactive effects; impacts of chronic (versus limited) exposure; dependence and withdrawal; species selection; food regulation; potential confounds from environmental enrichment; and chronic instrumentation and survival surgery.

Importance of Understanding Substance Use and Dependence Disorders

An initial IACUC consideration may be the justification of the use of animals to pursue knowledge of a disorder perceived by many to be “self-inflicted,” particularly when many abused drugs are obtained and used illegally. Unfortunately, many people unfamiliar with drug abuse and dependence fail to realize the complexity of the disorder and associate drug addiction with moral weakness and a lack of self-discipline. But substance abuse and dependence share characteristics with other chronic disorders such as heart disease and diabetes in that effective treatment may require lifelong attention to the vulnerabilities associated with it.

Clinical and preclinical research have demonstrated that genetic factors combined with environmental influences and drug-induced neurochemical changes can result in compulsive drug taking and an inability to discontinue use despite severe emotional, financial, and physical cost to the individual, the family, and the community. The total annual US economic cost of drug dependence has been calculated to exceed $600 billion, including health- and crime-related costs and loss of productivity, with two-thirds of those costs associated with use of the legal drugs alcohol and nicotine (Mark et al. 2008; Office of National Drug Control Policy 2004). Not reflected in these figures, however, are the indirect costs to society. Addiction and substance abuse are associated with an increased incidence of domestic violence, child abuse, and destruction of the family unit (Miller et al. 2006; Wells 2009). Addiction is also associated with increased public health risks such as cancer, heart disease, the spread of HIV/AIDS, and impaired driving (CDC 2005a,b).

A better understanding of the variables that contribute to substance abuse and dependence and the factors that make them so intractable is critical to improving public health and safety.

Need for Animal Models

Although in vitro studies can provide important information about molecular targets and cellular and/or systems effects of specific drugs, they are not predictive of relatively complex processes such as drug taking, physical dependence, tolerance, drug craving, and variables that increase vulnerability to relapse. Despite great strides in understanding addiction, as exemplified by the articles in this issue, much remains unknown about the neurobiology, genetics, and drug-behavior interactions that are relevant to preventing drug addiction. In fact, the National Institutes of Health (NIH) has long recognized the high priority of this research in its decades of funding for preclinical substance use and dependence research, primarily through the National Institute on Drug Abuse1 and the National Institute on Alcohol and Alcoholism.

Katherine L. Nicholson, DVM, PhD, is an assistant professor in the Department of Pharmacology and Toxicology and a member of the Animal Care and Use Committee at Virginia Commonwealth University in Richmond. Nancy A. Ator, PhD, is a professor in and the Director of the Division of Behavioral Biology in the Department of Psychiatry and Behavioral Sciences as well as Chair of the Animal Care and Use Committee at Johns Hopkins University in Baltimore, Maryland.

Address correspondence and reprint requests to Dr. Nancy A. Ator, Reed Hall B-122, Johns Hopkins School of Medicine, 1620 McElderry Street, Baltimore, Maryland 21205 or email ator@jhmi.edu.

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Thus the use of animal models as a complement to clinical research is essential to a systematic approach for analyzing the intertwined pharmacological, physiological, and behavioral components of the addiction process in relation to the concomitant neuroplastic changes that accompany and/or mediate the process (e.g., Reti et al. 2011). Given the strong role that the environment can play in substance abuse disorders (e.g., the initiation of drug taking), the use of animal models is also necessary to provide controlled conditions for assessing the variables that determine the effects of drugs that act in the central nervous system (CNS). Moreover, many types of studies involve numerous ethical considerations that deter the use of human subjects. Such considerations include concerns about promoting drug taking in naive individuals or relapse in those with drug dependence histories, exposing people to drugs with known health risks, and determining the effect of drug addiction on a developing fetus (e.g., Barr et al. 2011).

**Complexity and Procedural Diversity of Addiction Research**

As in all animal research, IACUC members assessing protocols related to substance abuse disorders must keep in mind the diversity of the models used (Lynch et al. 2010; Moser et al. 2011; Sanchis-Segura and Spanagel 2006). Research on substance abuse disorders includes assessment of neurochemical and neuroanatomical changes, physiological effects, and behavior. The complexity of the studies is increased by consideration of acute versus chronic drug exposure and the role of age and sex. Another layer of complexity is added because drugs from different pharmacological drug classes and with different mechanisms of action have unique profiles. One cannot assess a specific measure for cocaine and assume that it is possible to extrapolate those findings to the effects of alcohol or opioids or even to other CNS stimulants.

Despite overlap in some of the neurochemical effects of drugs that are abused (e.g., the general recognition that positive reinforcing effects are mediated by direct or indirect increases in dopamine levels in the nucleus accumbens; Koob and Bloom 1988; Volkow et al. 2010), the classes of drugs that are subject to abuse bind to different receptors and produce a range of effects. For example, opiates such as morphine bind to the mu opioid receptors, and ketamine binds to a receptor in the glutamate neurotransmitter system. The multiplicity of drug actions results in a wide range of assays that can be relevant to understanding the ways in which any one drug of abuse exerts its effects.

**Specific IACUC Concerns in Substance Abuse Research**

**Types of Drugs Studied and Legal Controls**

Drugs that are misused and abused as a function of their positive effects on mood or sense of well-being produce these effects through mechanisms in the brain. Such drugs are termed “centrally acting” or “psychoactive” (or “mind-altering,” in the case of hallucinogens). Not all psychoactive drugs are subject to abuse; exceptions include many antidepressants and drugs that treat schizophrenia.

Centrally acting drugs that are in development for clinical use must undergo assessment for abuse and dependence potential during the Food and Drug Administration (FDA) review process for safety and efficacy before approval for marketing (similar requirements are in place in the European Union and Japan). Such evaluations require submission of specific types of behavioral and other data from studies with laboratory animals and with humans (Ator and Griffiths 2003). A somewhat comparable FDA data review, including review of data from animals, takes place for instituting legal control of chemical entities (e.g., analogues of controlled drugs) that have been identified as problematic by the Drug Enforcement Administration.

Psychoactive drugs that have been deemed, via the governmental review process, to have abuse potential generally have greater legal controls than those that have not. That is, they are “scheduled” under national and international conventions by a process that began in the United States with the passage of the Controlled Substances Act in 1970. In the United States, psychoactive drugs that have no currently approved medical use (e.g., heroin and mescaline) are placed in the Schedule I category, whereas those that have an approved medical use (e.g., cocaine, ketamine, buprenorphine, and zolpidem) are placed in Schedules II–V based on an FDA evaluation of the particular potential for abuse. Alcohol and nicotine are excluded from this scheduling convention.

Investigators in the United States who conduct substance abuse research typically hold registrations as “Researchers” under the Controlled Substances Act as well as required state registrations and are thus legally approved to receive and use the controlled substances approved under their registrations. These individuals, not the institutions where they are employed, are personally responsible for meeting the federal or state legal requirements. Regulatory requirements for animal research investigators with federal registrations are fewer than for those who conduct research with humans or operate veterinary clinics, but all include a mandate for secure storage and for certain records of receipt and use of controlled substances.

**Sources of Drugs and Related Considerations**

Those who conduct federally funded pharmacological research typically obtain the compounds they need through NIH programs that supply them to grantees free of charge. Most compounds are supplied as powders, which do not expire if stored according to directions—obviously a very strong advantage. Researchers are well versed in methods for determining appropriate formulations and storage of

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2Most drugs are classified under a single schedule, but gamma-hydroxybutyrate (GHB) is in both Schedule I and, because it is approved as a treatment for narcolepsy, Schedule III.
solutions or suspensions for subsequent administration by the route(s) necessary for each type of study. For example, a solution to be given intravenously normally requires filter sterilization before delivery, whereas one for oral administration does not.

Drug dose typically must be varied across a wide range to fully characterize the drug’s effects in the particular paradigm. Because these dose-effect evaluations are the gold standard of pharmacology research, a drug formulation that is commercially available for clinical use would be appropriate for experimental use only in rare circumstances. Generally, a commercial formulation must be diluted (because volume of injection is considered an important variable to hold constant), thereby obviating the assumed value of using the commercial solution. Furthermore, commercial solutions often have preservatives that are undesirable for many research applications and make it difficult to achieve an appropriate vehicle control solution.

For compounds not available through the NIH drug supply program, commercial sources are usually companies that have a long-standing reputation for providing high-quality compounds for use in research. These supplies typically come with documentation describing the purity and other characteristics of each compound, as well as technical information about its appropriate storage and preparation.

Psychoactive Drug Effects

IACUC members and animal care staff must be aware that substance abuse research involves administration of drugs for which effect(s) may or may not be predictable at the time of protocol review. Conversely, a principal investigator (PI) may be using psychoactive drugs that are already well characterized in a variety of non-abuse-related studies (cf. Reti et al. 2011) or that perhaps are serving as a standard comparator in a study with a new compound or new assessment.

Although certain drugs may be seen as dangerous or life threatening when abused by humans, they should not pose a threat to an animal’s health or well-being when administered by personnel who are knowledgeable about appropriate dose ranges and dosing conditions. In fact, direct effects on the animal may well be pleasurable, given that animals have been shown to self-administer the same drugs that humans abuse even in the absence of any conditions of dependence (Ator and Griffiths 2003). The vast majority of abuse-related research strives to use dose ranges that fully characterize behavioral effects without being life threatening, particularly studies that include behavioral training (e.g., self-administration, drug discrimination, conditioned place preference; Morrison et al. 2002).

The protocol should describe anticipated effects related to the dependent measure of interest as well as potential adverse effects related to the drugs used. In addition to potential toxicity, the most relevant effects are those that affect food and water consumption, mobility, and sedation.

Chronic Drug Exposure and Physical Dependence

Chronic Exposure

Drug abuse disorders in humans are associated with a number of distressing and/or unpleasant sequelae, particularly after chronic and/or compulsive drug administration. These effects are essential components of the disorder and must be understood in order to develop treatments for prevention or alleviation. Whereas initially it was believed that the euphoric effects produced by drugs of abuse drove compulsive drug taking and an inability to “quit,” scientists now understand that the aversive aspects of drug cessation play an important, perhaps essential, role in continued drug taking (Gardner 2011; Volkow et al. 2010).

The use of limited-exposure procedures is excellent for answering many questions relevant to substance abuse disorders, and many drug abuse studies specifically design the dosing regimen to avoid the development of neuroadaptive changes such as tolerance and dependence. However, such a design may not adequately model all aspects of human drug use patterns. Researchers interested in understanding the addiction process, and particularly those engaged in developing pharmacological treatment for substance abuse, are turning to models that include the development of physical dependence and/or promote escalating drug intake (for review, see Ahmed and Kenny 2011). Although these methods may entail more stress for the subjects, they also more appropriately simulate the human condition and result in a more powerful model for understanding underlying neurochemical changes and for improving the predictive power of studies to examine potential treatments.

One animal welfare concern for models that use chronic and/or unlimited drug access, through either self-administration or investigator-administered methods, is the health and well-being of the subjects during dosing. This is particularly relevant for subjects with extended access to drugs that may result in reduced food intake and/or problems with digestion (e.g., constipation produced by opioids and anorectic effects of psychostimulants). A monitoring plan should be in place for assessing behavior, appearance, elimination, and general levels of food consumption along with a contingency plan for addressing adverse events during chronic drug exposure. Good coordination with animal care staff is essential in these and most types of studies involving psychoactive drugs.

Dependence and Withdrawal

A further concern is the impact of drug cessation, which results in a spontaneous withdrawal or abstinence syndrome in physically dependent animals. In fact, the appearance of this syndrome is the evidence that physical dependence has developed (Ator and Griffiths 2003). The probability of an abstinence syndrome after chronic drug administration is not limited to abused drugs. For example, withdrawal syndromes
have been associated with nonabused drugs such as selective serotonin reuptake inhibitors (Rosenbaum and Zajecka 1997); extensive exposure to psychostimulants, on the other hand, is associated in humans with psychological rather than physical dependence.

The development of drug dependence is a function of dose, frequency, and duration of administration. Physical dependence reflects the cellular changes that occur in response to chronic drug exposure as the body attempts to create a new state of homeostasis (Bailey and Connor 2005; Henningfield et al. 2009; Wahlstrom 1979), but the neuroadaptive changes necessary for dependence development require protracted periods of adequate blood levels and receptor occupancy. Thus many studies that use single daily dosing, as with induction of sensitization or drug discrimination procedures, are usually insufficient to induce dependence and do not result in a withdrawal syndrome when the drug delivery is stopped.

It is also important to understand that withdrawal syndromes differ across pharmacological classes. Although opioid withdrawal is often characterized by unpleasant sequelae such as diarrhea, malaise, and rhinorrhea, these side effects are not typically life threatening (Higgins and Sellers 1994). In contrast, withdrawal from CNS depressants such as ethanol, benzodiazepines, and barbiturates causes anxiety and elevated blood pressure, and in the case of ethanol and barbiturates can cause potentially life-threatening seizures (Little 1991; Pinel 1980). Nicotine and cannabis withdrawal, on the other hand, frequently produce only subtle signs. Animal models involving physical dependence on these drugs may necessitate the use of highly sensitive measures, such as rate of responding for food reinforcement, to assess withdrawal (Malin 2001; Malin and Goyarzu 2009; Ramesh et al. 2011).

For studies designed to produce physical dependence as evaluated by withdrawal, IACUC members should consider the constellation of signs associated with a particular pharmacological class of drugs, the unit dose, and dosing frequency and duration before automatically designating the study as one that produces unalleviated pain or distress. When a withdrawal syndrome is likely, the protocol should describe the expected characteristics and duration along with any approaches to mitigate discomfort (e.g., through readministration of the dependence-producing drug).

A final consideration is disposition of subjects following protracted exposure to drugs of abuse. For drugs known to produce a withdrawal syndrome after particular schedules of delivery, the gradual reduction (tapering) of the drug dose can prevent or reduce drug withdrawal effects at the termination of the study. This method is often advised with some drugs in human patients.

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3Free access to water may enhance the severity of dependence and withdrawal, but the dosing regimen should be planned to prevent inadvertent spontaneous withdrawal.

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Species Selection

As in all animal research, the appropriate choice of species is an important consideration. Rodents often can serve as subjects in studies that address drug addiction, but they cannot replace nonhuman primates in all cases. A number of differences in neurotransmitter systems exist between primates and rodents in regions relevant to abuse-related behaviors. For example, comparison of dopaminergic receptors across species has shown that distribution in the frontal cortex and the hippocampus is most similar among primates and different from rodents (Goldman-Rakic et al. 1992; Weerts et al. 2007).

The pharmacokinetic profile of a drug is especially important in self-administration studies, as investigators have demonstrated that differences in biodisposition can affect self-administration behavior among drugs with otherwise similar pharmacological effects (Meisch 1987; Winger et al. 2002). Pharmacokinetic studies in humans, monkeys, and rats have shown that monkeys provide the most qualitatively and quantitatively accurate predictions of human pharmacokinetic parameters compared with other species, a finding that may be particularly important for studies of dependence and dependence potential (Ward and Smith 2004a,b).

Investigators may build on findings from published studies with the same species to assess current results in the context of existing data. In all cases, of course, the PI will need to provide the rationale for the particular species chosen for the study.

Food Regulation

Regulation of food intake is often used in drug abuse-related preclinical research to motivate subjects to respond in behavioral training procedures (Morrison et al. 2002). Food restriction can also promote drug self-administration, which is itself an important phenomenon to understand (Cabeza de Vaca and Carr 1998; Carr 2002; Macenski and Meisch 1999).

In addition, some substance abuse-related research directly assesses food intake as influenced by drug administration. Examples are studies that have examined the role of the endocannabinoid system on food consumption (Gomez et al. 2002; Scopinho et al. 2011).

For studies that may take many months to complete, maintenance of adult animals at a constant weight is a means to control for total dose of drug received throughout the study. Although most studies specify doses in terms of mg/kg of body weight, in some circumstances the total amount of drug received is a critical variable. Such measurements are essential for nonhuman primates, which may vary significantly in body weight and composition, and for rats, which can gain significant amounts of weight relatively rapidly under free-feeding conditions.

4Free access to water is common in food restriction protocols.
All protocols that use food regulation should include the following: (1) justification for the level and duration of restriction, (2) a description of how the restriction will be arranged and whether individual housing is required, and (3) a plan for monitoring body weights.

Induction of Stress

Numerous investigators have linked substance abuse disorders in humans to stress (Belujon and Grace 2011; Breese and Blendy 2010; Melis et al. 2009). In this issue Hopf and colleagues (2011) address this link in a comprehensive manner in relation to alcohol drinking.

In general, stress has been seen as promoting initial drug use in humans as well as relapse in abstinence patients (Breese et al. 2011; Enoch 2011). Therefore, in animal models, particularly those examining drug taking and relapse (e.g., reinstatement models), stress may be an integral component of the study and may require appropriate pain categorization. Induction of stress is often accomplished by the use of inescapable footshock (Barsy et al. 2011; Shaham et al. 2000), restraint (Le et al. 2009; Mantsch et al. 2007), or administration of chemical mediators of stress such as cortisol or other chemicals that stimulate the hypothalamic-pituitary-adrenal axis (Anker and Carroll 2010; Shalev et al. 2010). Some studies use shock to alter levels of behavioral responses. For example, it may serve either as a punisher to decrease responding or as a negative reinforcer to train an avoidance response (e.g., as an alternative to food reinforcement in operant procedures in which drug effects may alter food motivation, such as testing of stimulants that have anorectic effects; Li et al. 2010; Morrison et al. 2002; Raffalli-Sebille and Chapouthier 1991). In such situations, the shock is escapable and/or avoidable and thus constitutes no more than momentary pain/distress.

Investigators using footshock and other stressors should include the following information in the protocol: (1) justification for the use of the stressor, (2) duration of the stress exposure, (3) plans for monitoring animal health, and (4) a description of the procedure to ensure that the equipment generates the appropriate shock intensity.

Environmental Enrichment

The use of environmental enrichment is encouraged for all species to enhance well-being. When the environment is not coordinated with the research design, however, enhancement can alter both behavioral and neurochemical results in some studies of psychoactive drugs (Chauvet et al. 2009; Perry et al. 2008; Stairs et al. 2006; Thiel et al. 2009; Wooters et al. 2011).

For humans, enhanced environments and positive social interactions are sometimes associated with a decrease in substance abuse and diminished relapse (Solinas et al. 2009, 2010). Thus for animals social housing and the presence of multiple types of items in a cage can present important confounds in certain types of substance abuse research. Individual housing may be necessary to minimize environmental complexity by eliminating social interactions (Bardo et al. 2001; Thiel et al. 2010), and investigators may also request exclusion from enrichment programs or control of the types and frequency of toys and food treats. Although they should provide justification for this exemption, the request should not be considered unusual.

Chronic Instrumentation and Survival Surgery

As in many other types of preclinical studies, substance abuse research may involve survival surgery procedures and chronic instrumentation for drug administration or testing purposes. Consistent with all surgical protocols, it is important for the protocol to include information about anesthesia and intraoperative monitoring, methods for ensuring sterility, surgical procedures, and postoperative care, including use of analgesics.

Substance abuse models may deviate from other disciplines in the number of surgeries performed and potential confounds from the type of postoperative analgesia. For example, the use of opioid analgesia may be precluded in studies designed to examine abuse-related effects of opioids (e.g., tolerance), necessitating the use of another class of analgesic agent.

Animals in repeated or continuous drug administration or self-administration studies may require the surgical implantation of a venous or intragastric catheter or some form of extended release device (minipumps, microinfusion pumps, drug pellets). The extended release device obviates the need for numerous injections and is thus less stressful for the animals. Catheters may remain in place and patent for several months (rats) to many months (nonhuman primates). Investigators should describe the care and maintenance of these catheters and their exit sites.

Many long-term studies entail a within-subjects design, which minimizes total animal numbers while increasing the statistical power of comparisons, typically critical for the design of self-administration studies, whether with rodents or nonhuman primates. To achieve the desired goal, it may be necessary to remove and/or replace the catheter or extended release device. For catheters, if patency is lost before completion of the study, an investigator may decide to recatheterize rather than remove the subject from the study and substitute a new one. Venous catheterization is a relatively minor surgical procedure, and the catheterization of multiple veins permits (1) an overall reduction in the number of animals, (2) a behaviorally experienced subject to complete a study, and (3) assessment of drug history as a variable.

The implantation of intracranial cannulae or of electrodes for intracranial self-stimulation can be considered a minor surgical procedure because it is minimally invasive and of relatively short duration. Postoperative pain is treatable with topical analgesics on the suture line and/or a nonsteroidal.
anti-inflammatory drug. As with catheters, the protocol should include a description of the plan for long-term maintenance of these implants.

Conclusion

As the articles in this issue illustrate, preclinical research in the area of drug addiction involves a number of research questions and a range of procedures. The following issues often concern IACUC members:

- the nature of the drugs to be studied, their sources, and their regulatory control;
- concern about whether the drug itself will produce discomfort for the animal;
- assessment of adverse effects of the drugs on the animals (e.g., deterioration of normal behavior);
- attention to the effects of drug discontinuation on the animal (withdrawal symptoms);
- validity of requests for exemption from social housing and certain types of environmental enrichment, and
- multiple surgical procedures in the course of the study.

We have addressed aspects of these concerns by placing them in the context of the nature and design of the research itself. As in other types of research, investigators will need to provide evidence of appropriate training and experience in drug addiction research.

Although these types of studies may pose special considerations for IACUC review, research to better understand the variables that converge to produce drug addiction and that make cure so elusive is of compelling national significance, and the use of animal models will continue to be essential to this endeavor.

References


