

Animal Models of Drug Addiction in Support of Novel Therapeutic Strategies

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Addictions to a variety of substances both licit (e.g., alcohol, nicotine) and illicit (e.g., marijuana, cocaine) are a pervasive national and international social and economic challenge, accounting for as much as \$600 billion annually in cumulative losses in the United States (cited in Nicholson and Ator 2011). The treatment of addictions and addictive behaviors is thus an important public health concern. Basic animal studies have greatly contributed to progress in this area and will surely continue to yield significant insights into the neuroanatomical circuitry, neurophysiological function, neurochemical changes, and behavioral processes underlying addiction.

Current clinical neurobiological methods such as brain imaging have expanded knowledge and provided novel insights into the most complex human brain-behavior interactions. The science of addiction is revealing that alterations of brain-behavioral processes can have a complex mixture of intrinsic and extrinsic causes. A better understanding of the etiology and brain mechanisms directly involved will provide more effective addiction prevention and treatment approaches.

The topic is critically important and broad enough to warrant two issues, in which experts review efforts both to understand a variety of substance dependences and to develop therapeutic treatments for them. In this issue the authors address addictions to alcohol, nicotine, marijuana, opiates, cocaine, methamphetamine, and ketamine. Articles in the subsequent, companion issue will focus on the neurobiology of addiction-like behaviors, addressing addiction and psychiatric disorders, sex differences in addiction, and various aspects of food addiction. Each issue also includes an article on IACUC considerations relevant to addiction-related research.

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Overview

Substance addiction is a chronic relapsing brain disorder that results in the addicted individual's inability to limit drug consumption despite detrimental consequences (Meyer 1996). Although initially an individual's substance use is voluntary, with repeated use the brain's reward system is commandeered and neuroadaptive alterations render the individual unable to withstand the irresistible urges to use substances and therefore chronically susceptible to relapse. The slippery slope of drug addiction may start with experimentation or social use to experience the positive reinforcing (euphoric) effects, and then spirals downward in a pattern of abuse—from escalating compulsive drug seeking and taking in an attempt to alleviate the undesirable negative effects (e.g., dysphoria, anxiety, stress), to dependence, and then to withdrawal and abstinence, during both of which relapse to compulsive use is likely (Koob and Le Moal 1997).

Understanding vulnerability to relapse as an integral part of addiction is crucial to the effective treatment of drug addiction (Leshner 1997). Much addiction research therefore focuses on efforts to (1) elucidate the induced neuroadaptations in the reward and other brain systems, particularly those that mediate the transition from controlled to uncontrolled drug use; (2) determine individual differences in behavioral and/or neurobiological vulnerabilities that promote substance dependence and relapse; and (3) identify pharmacological targets for treatment and relapse prevention. Environmental stressors contribute an additional layer of complexity (Nicholson and Ator 2011 describe the layers of complexity in addiction research with animals).

The translational articles in this issue present animal models of addiction in a highly integrated way with the latest clinical findings to convey the current understanding of the effects of alcohol and drugs of abuse on brain structural and functional processes that are linked with behavioral outcomes. Such research is critical to efforts to develop novel approaches and insights for treatment.

Alcohol, Nicotine, and Marijuana

Alcohol

Hopf and colleagues (2011) examine the interaction of alcohol addiction and stress. They document the human literature on

various aspects of alcohol consumption and the contributions of stress to the development of alcoholism, and survey the animal research literature on the neurobiological and molecular bases of alcohol intake-stress interactions. Specifically, the authors discuss (1) the roles of acute stress and negative affect in relapse and enhanced alcohol use; (2) escalating compulsive alcohol intake despite detrimental consequences; (3) molecular neuroadaptations that contribute to stress-alcohol cross sensitization; and (4) efforts that target stress via behavioral modification strategies as potential therapeutic interventions for alcohol addiction.

Hopf and colleagues make the case that:

- Animal models of alcohol addiction have been invaluable in revealing the molecular basis of pathological alcohol-related behaviors (binge drinking, dependence-induced alcohol intake, aversion-resistant alcohol intake, alcohol-induced stress modulation, and the consequences of prior stress-alcohol exposure on subsequent susceptibility to stress and alcohol intake);
- It is of paramount importance to improve and expand animal models that have yielded important neuroadaptive clues that would be virtually impossible to glean from human studies and that will support the development of innovative behavioral and pharmacological therapies; and
- Novel animal models that mimic more complex aspects of human alcoholism remain to be developed.

Nicotine

Sobrian and Holson (2011) assess the developmental consequences of pre- and neonatal nicotine exposure in the rodent model, in the context of the extensive research on the effects of early nicotine exposure in humans.¹ A thoughtful description of design considerations appears at the end of this article.

The incidence of maternal smoking during pregnancy is high and quite likely growing worldwide, although developmental exposure to nicotine is one of the most preventable causes of injury to the developing fetus (Bruin et al. 2010). The authors consider the following most frequently reported effects of maternal smoking during pregnancy on human offspring: (1) decreased body size and subsequent obesity; (2) cognitive deficits; and (3) attention deficit/hyperactivity disorder. But they also cite studies with confounding variables that in some cases either statistically negate the results or are found to be directly responsible for the effects attributed to maternal smoking during pregnancy.

¹Although cigarette smoke contains more than 4000 ingredients, hundreds of which may be teratogenic, investigations have focused on nicotine, the single most important factor in triggering negative effects on neurodevelopment (Ernst et al. 2001; Slotkin 1998) and the causal agent in intrauterine growth retardation associated with prenatal nicotine exposure (Somm et al. 2009). Sobrian and Holson therefore address the effects of developmental nicotine exposure in animals, while acknowledging that other cigarette smoke ingredients may also contribute to adverse developmental outcomes in nicotine-exposed humans.

Certain variables can be eliminated or mitigated only in animal models of ontogenetic exposure to cigarette smoke/nicotine in which the molecular mechanisms of perinatal exposure can be linked to specific outcomes. Sobrian and Holson therefore compare animal models of nicotine exposure (Buka et al. 2003; Cornelius et al. 2005; O'Callaghan et al. 2009; Roberts et al. 2005) and note that measures of nicotine-induced cognitive deficits, alterations in drug challenge responses, psychiatric problems, and early physical and reflex developmental alterations largely accord with those of human epidemiological studies. However, the authors repeatedly cite conflicting results and conclude that "no consistent phenotype emerges as a result of gestational or developmental exposure to either cigarette smoke or nicotine."

The authors provide three plausible explanations—all associated with decreased statistical power—for the large number of rodent studies that fail to duplicate probable effects of gestational nicotine exposure in humans: (1) inadequate sample sizes, (2) sample size inflation due to the use of individual pups from a litter as the unit of statistical analysis, and (3) inclusion of replicates in experimental design.

Clear, reproducible outcomes and the identification of more sensitive and widely used behavioral measures of the effects of developmental nicotine exposure are necessary to ensure the translational value of experiments with animals.

Marijuana

Ramesh and colleagues (2011) note that in the United States marijuana (*Cannabis sativa*) is the illicit drug most widely consumed by teenagers and adults (Johnston et al. 2010a,b), and they speculate that this consumption pattern may be due in part to the public perception that marijuana lacks dependence liability and that the symptoms of cannabis withdrawal are not particularly severe (Mansbach et al. 1996). But the medical community has begun to accept that marijuana-induced dependence represents a public health concern as human studies suggest that withdrawal symptoms—anger, aggression, irritability, anxiety, anorexia, restlessness, and sleep difficulties with strange dreams (Budney and Hughes 2006; Tsou et al. 1995)—may contribute to continued use (Moore and Budney 2003).

The authors review information about cannabinoid receptors and their actions. The primary psychoactive component of *C. sativa*, Δ^9 -tetrahydrocannabinol (THC; Gaoni and Mechoulam 1964), other phytocannabinoids (Elsohly and Slade 2005), the endocannabinoids N-arachidonyl ethanolamine (anandamide) (Devane et al. 1992) and 2-arachidonylglycerol (Mechoulam et al. 1995; Sugiura et al. 1995), and a host of synthetic cannabinoids bind to and activate two types of cannabinoid receptors.

The authors describe animal models used to evaluate potential pharmacotherapies to treat cannabis dependence and withdrawal; compounds investigated include cannabinoid substitutes (THC), inhibitors of endocannabinoid catabolic enzymes (fatty acid amide hydrolase) and monoacylglycerol

lipase (Schlosburg et al. 2009), and noncannabinoid drugs (lithium and clonidine) (Cui et al. 2001; Lichtman et al. 2001). Ramesh and colleagues express concern about the sustainability of using THC substitution therapy to attenuate cannabis withdrawal symptoms in marijuana-dependent humans because the compound “is also primarily responsible for marijuana’s pharmacological effects.” What remains to be determined in clinical trials is whether endocannabinoid catabolic enzyme inhibitors are efficacious in attenuating withdrawal in marijuana-dependent humans.

Cocaine

Ahmed and Kenny (2011) present evidence that research is advancing toward “cracking the molecular code of cocaine addiction,” illustrating the importance of designing and testing the validity of animal models to shed light on the molecular neurobiology of cocaine addiction. The authors summarize recent research in rats, the most frequently used animal species in drug addiction research, and the behavioral features associated with cocaine addiction that can be selectively induced after extended (but not after limited) access to cocaine for self-administration.

They observe that a critical level of drug self-exposure may be required to induce addiction in most animals and that there are three hallmark features of cocaine addiction: (1) gradually escalating cocaine use, (2) escalating drug seeking despite adverse consequences, and (3) increased susceptibility to stress- and drug-primed craving.² The authors note, in particular, that animals exposed to extended (versus limited) access develop working memory deficits that may exacerbate their inability to regulate cocaine use. Loss of control over cocaine intake (as assessed by continued drug taking despite the opportunity to choose more natural rewards) is an indicator of addiction.

Ahmed and Kenny use the term “addiction-like” (referring to behavioral changes evocative of some symptoms of cocaine addiction) as an acknowledgment that mere cocaine self-administration or reinforcement is necessary but insufficient evidence of an addictive phenotype in nonhuman animals (Belin et al. 2008; Deroche-Gamonet et al. 2004).

Finally, the authors report that a comparison of rats exposed to extended versus limited cocaine access (Ahmed 2010) revealed the existence of a dorsal striatal pathway that is altered in cocaine-addicted humans and that selectively regulates cocaine intake. This pathway consists of homeostatic interactions between microRNA and important regulators of neuroplasticity, methyl CpG binding protein 2 (MeCP2) and brain-derived neurotrophic factor, a discovery that provides an entirely novel direction for the development of effective antiaddiction pharmacotherapies.

²Although drug craving is not a current diagnostic criterion of addiction in the American Psychiatric Association *Diagnostic and Statistical Manual of Mental Disorders* (APA 1994), the authors and others (e.g., Miller and Holden 2010) expect that it will be included in the next revision.

Opiates

In three complementary papers, Reti and colleagues (2011), Barr and coworkers (2011), and Glass (2011) address different aspects of opiate addiction.

Reti and coauthors describe current molecular approaches to understanding synaptic plasticity through the actions of neuronal activity–related pentraxin (Narp). They detail the possible direct contribution of Narp and related proteins to brain changes that influence long-term behavioral effects induced by drugs of abuse and discuss the possible role of Narp in modulating aversive conditioning. Because Narp activation is induced in brain regions associated with aversive conditioning after morphine withdrawal, it may mediate changes in neuroplasticity associated with chronic opiate use and withdrawal. The authors also discuss the relevance of Narp knockouts in studies of the mechanisms of extinction of drug craving.

The article by Barr and colleagues focuses on the important clinical issue of prenatal opiate exposure, contrasting human and animal data to illustrate the effects of repeated opiate exposure on various brain systems in a developmental context and on tolerance to and dependence on opiates. Infants exposed to opiates in utero or postnatally (e.g., via breast milk or pain medications) can develop physical dependence as evidenced by physical symptoms of opiate withdrawal.

Barr and colleagues highlight systems and other neurosubstrates (e.g., the *N*-methyl-*D*-aspartate [NMDA] glutamatergic system and its influence on nitric oxide, the protein kinase signaling cascade, substance P, endothelin, and acetylcholine) that may be involved in the development and expression of morphine-induced tolerance, dependence, and/or withdrawal. The opioid system of the human newborn is functionally different from that of the adult, and the biology of the developing central nervous system contributes to the challenge of evaluating and treating opiate-induced changes in the newborn. The authors describe specific behaviors that have been identified to gauge the severity of neonatal withdrawal symptoms.

Glass explains the contributions of the NMDA receptor system both to opioid dependence and withdrawal and to learned aversion, and indicates that NMDA receptors may be a useful target for addiction treatment. Sensory, limbic, and memory processes contribute to drug dependence, and pharmacological agents that target them are often suboptimal because of their own abuse potential or side effects. Focusing on the ionotropic NMDA-type glutamate receptor as a potential substrate for addiction treatment, Glass cites seminal genetic studies and clinical reports that support the role of NMDA receptors in opioid addiction in humans. He also describes animal studies used to investigate the involvement of NMDA receptors in opioid dependence and to establish a relationship between NMDA and mu-opioid receptor systems. The physical symptoms of opiate dependence and withdrawal involve sensorimotor, autonomic, and neuroendocrine systems, and studies demonstrate the potential of NMDA receptor antagonists to alleviate these physical symptoms in animal models and humans.

Methamphetamine

Kuhn and colleagues (2011) address the neurotoxic effects of methamphetamine (Meth) on the brain, observing interesting heterogeneities in damage and recovery. Specifically, they note that the nucleus accumbens (NAc) is highly resistant to Meth-induced neurotoxicity compared with the caudate-putamen (CPu).

A member of the amphetamine family of psychostimulant drugs, Meth causes a persistent neurotoxic assault on the brain's dopamine (DA) neuronal system that manifests as DA depletion, tyrosine hydroxylase inhibition, DA transporter inactivation, decreased vesicle monoamine transporter function, fine unmyelinated axon degeneration, and apoptosis (Jayanthi et al. 2002). The authors cite data indicating staggering economic, medical, legal, and societal costs of widespread Meth abuse and addiction (Rand 2009). The NIH National Institute on Drug Abuse,³ the White House Office of National Drug Control Policy, and the US Department of Justice's Drug Enforcement Administration classify Meth abuse and addiction as an epidemic; and the United Nations Office on Drugs and Crime reports that the use of amphetamines, including Meth, has eclipsed that of cocaine and heroin worldwide.

In addition to the NAc and CPu, the authors review Meth-induced neurotoxicity in the substantia nigra, hippocampus, and cortex, and they discuss a gradient of susceptibility to amphetamine-induced toxicity in the ventrolateral CPu, which shows greater neuronal damage (Harvey et al. 2000) and microglial activation (Thomas et al. 2004) than the medial CPu. This difference may be related to the heterogeneous distribution of DA uptake and release sites in the CPu, and could also be influenced by local production of neurotrophic factors such as pleiotrophin. Meth-induced neuronal damage in other brain regions is less dramatic, with substantia nigral and ventral tegmental area dopaminergic neurons generally demonstrating limited (Brown et al. 2006) or no neuronal loss (Thomas et al. 2009).

Kuhn and colleagues review processes that may mediate Meth-induced damage to the brain dopaminergic system, and discuss the role of nonneuronal cells (microglia and astrocytes) in mediating Meth-induced neurotoxicity. They also suggest that the pattern and extent of the neurotoxicity may be a function of brain-region-specific cross talk among nerve endings, microglia, and astrocytes, and may depend on subsequent production of proinflammatory cytokines, prostaglandins, and reactive oxygen and nitrogen species (Hanisch 2002).

Considering the very important roles of the NAc in mediating reward, the addictive properties of drugs of abuse, and executive functions, the authors opine that perhaps it is fortunate that the NAc is resistant to Meth neurotoxicity.

Ketamine

Trujillo and coauthors (2011) provide a comprehensive overview of ketamine—its pharmacology, neurobehavioral effects,

and medical uses; its neurochemical and neuroadaptive impacts on glutamate receptor function; and its euphorogenic effects that contribute to its abuse and addiction potential. They specifically describe the drug's anesthetic profile, analgesic effects at a variety of doses, ability to mimic symptoms of schizophrenia, antidepressant effects, and rewarding effects. But further research is necessary to identify the systems that are responsible for these characteristics.

Ketamine has gained popularity especially among young people, and Trujillo and colleagues express concern about this trend because of the drug's potentially dangerous dissociative state, which has led to adverse consequences such as burns, falls, drowning, traffic accidents, and vulnerability to sexual assault (Dillon et al. 2003; Freese et al. 2002; Jansen 2000; Smith et al. 2002).

Ketamine also attenuates the behavioral effects of psychostimulants, producing a dose-dependent decrease in stimulant-induced locomotor activity (Uzbay et al. 2000) even at doses that usually produce activation. In light of the locomotor depression observed at low doses in animals, and the fact that ketamine simulates the symptoms of schizophrenia, the authors posit that animal studies with this drug may "more accurately reflect the clinical research and lead to a better animal model of schizophrenia."

Concluding Thoughts

The articles in this issue highlight the remarkable advances in the field of addiction research, drawing from a broad array of studies that have used animal models to elucidate brain mechanisms, structural and functional alterations, and associated behavioral changes. The strength and significance of these studies lie in the accuracy with which they model the clinical condition and thus contribute to effective translation. The authors also note some of the challenges and opportunities in the science of drug abuse and treatment of addiction.

Given the complexity of addiction, combined approaches incorporating multi- and transdisciplinary methods and strategies are needed to ensure progress in treatment and prevention. Therapeutic strategies must consider the complexity of the brain, the multiplicity of processes and systems affected by alcohol and drugs, the diverse biobehavioral processes involved in use, abuse, and addiction, and the complicating effects of individual differences.

Clinical neuroscience and human neurobehavioral investigations are providing essential, novel, and complementary insights that continually advance understanding of this widespread public health challenge. It is our sincere desire that readers will become "hooked" on the fascinating and important possibilities described in this and the next issue of the *ILAR Journal*.

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