Cracking the Molecular Code of Cocaine Addiction

Serge H. Ahmed and Paul J. Kenny

Abstract

Cocaine addiction is a behavioral disorder defined by behavioral symptoms that set it apart from nondisordered forms of drug use. Here we review evidence in rats (the most frequently used animal model in the field) that it is possible, after extended (but not after limited) access to cocaine for self-administration, to selectively induce some of these behaviors: gradual escalation of cocaine intake, enhanced motivation for the drug despite increased costs (or negative consequences), and increased sensitivity to drug- and stress-primed craving-like behavior. Animals with extended drug use also present selective neurocognitive deficits (e.g., compromised working memory) that may impair their ability to regulate cocaine intake. In some rats, extended access to cocaine for self-administration is associated with loss of control over cocaine intake, as assessed by continued drug use despite the opportunity to make a different choice and to the exclusion of more natural and rewarding activities. These rats may represent the most advanced and severe stage on the path to cocaine addiction. Finally, comparisons of rats with extended versus limited access to cocaine for self-administration have recently revealed the existence of a new molecular pathway in the dorsal striatum (a brain region altered in cocaine-addicted humans) that causally and selectively controls cocaine intake. This pathway involves unforeseen homeostatic interactions between microRNAs (a class of nonprotein-coding RNAs) and some key molecular regulators of neuronal plasticity (e.g., MeCP2 and BDNF). This discovery provides an entirely new direction for the development of effective antiaddiction treatments.

Key Words: addiction; brain-derived neurotrophic factor (BDNF); cocaine; heroin; methamphetamine; methyl CpG binding protein 2 (MeCP2); miR-212

Introduction

Research involving animal models of cocaine addiction can be conceptualized as reverse addiction "treatment." Contrary to clinicians who seek to help cocaine-addicted people to become and remain abstinent, researchers who work with animals seek to make drug-naïve subjects dependent on or addicted to cocaine. The goals of this research are to gain a better understanding of the neurobiology of cocaine addiction and, ultimately, to use this knowledge for treatment development.

Until recently, it was generally believed that making animals addicted to cocaine was relatively easy—that it would suffice to expose animals to cocaine (the "disease-causing agent") to induce addiction or at least vulnerability to it. Then the comparison of cocaine-exposed animals to drug-naïve controls would shed light on the neuropathological changes that are hypothesized to underlie cocaine addiction (Ahmed 2010).

Over the past 25 years, this paradigm has inspired a flurry of research that identified many cocaine-induced changes in the brain, from the molecular intracellular level to the circuit level. However, without independent and valid evidence for addiction-like behavior in cocaine-exposed rats, it is often difficult to interpret unambiguously these numerous changes in terms of addiction-causing neuropathological dysfunctions (Ahmed 2010). This difficulty may partly explain why, despite much progress in understanding the neurobiology of cocaine actions (e.g., cocaine-induced reinforcement), research involving animal models of cocaine addiction has so far had little significant translational impact (Koob et al. 2009). Most advances in current treatments for cocaine addiction still come from the "bedside," not from the laboratory bench.

Fortunately, this situation is changing. Many researchers in the field now acknowledge that cocaine self-administration or reinforcement alone is necessary but not sufficient evidence for an addiction-like profile or phenotype in nonhuman animals (Ahmed and Koob 1998; Belin et al. 2008; Deroche-Gamonet et al. 2004; Negus 2006; Vanderschuren and Everitt 2004; Wolffgramm 1991; Wolffgramm and Heyne 1995). Such recognition directly follows from a greater awareness of the medical diagnosis of cocaine addiction, which is distinct from nondisordered forms of cocaine use (e.g., occasional or controlled use) (Edwards and Gross 1976; Martin et al. 2008; Saunders 2006).

To be considered valid, animals must, in addition to self-administering cocaine, develop or present an array of behavioral

Serge H. Ahmed, PhD, is Research Director at the Institut des Maladies Neurodégénératives at the Université Bordeaux-Segalen in France. Paul J. Kenny, PhD, is Research Director at the Scripps Research Institute in Jupiter, Florida.

Address correspondence and reprint requests to Dr. Serge H. Ahmed, Institut des Maladies Neurodégénératives, Université Bordeaux-Segalen, CNRS-UMR 5293, 146 rue Léo-Saignat, 33076 Bordeaux, France or email sahmed@u-bordeaux2.fr.

changes that recapitulate important behavioral features of cocaine addiction in humans (e.g., escalation of cocaine intake, continued drug use despite negative consequences). Attempts to apply these criteria reveal that making animals addicted to cocaine is possible but less straightforward and more difficult than previously thought (Ahmed 2010), a conclusion that is in keeping with both the difficulty of managing cocaine addiction and, indeed, almost everything known about this behavioral disorder in humans.

Systematic comparisons of rats with an addiction-like phenotype to drug-exposed but nonaddicted controls have recently led to identification of specific brain molecular regulators that causally and selectively control escalation of cocaine intake. Escalation to heavy cocaine use is a well-established hallmark stage in the transition to addiction that can be reliably and selectively induced in animals through extended (but not limited) access to the drug (extended access to cocaine may entail 6 hours of access per day for several days; limited access, 1 hour per day for the same period).

The creation and testing of valid animal models of cocaine addiction, though initially time-consuming, are necessary for the discovery of new insights into the molecular neurobiology of cocaine addiction and, possibly, new directions for treatment development (Hollander et al. 2010; Im et al. 2010). We mainly focus on the effects of extended access to cocaine for self-administration on both behavior and the brain, but also occasionally cite relevant research on other drugs of abuse, such as heroin and methamphetamine.

First we summarize recent research in rats—by far the most frequently used animal species in experimental addiction research (Ahmed 2010)—showing that important behavioral features of cocaine addiction can be relatively selectively induced in animals after a history of extended, but not limited, access to cocaine for self-administration. Then we describe how systematic comparisons between animals with a history of extended access to cocaine and controls with limited access have revealed the existence of a new molecular pathway in the dorsal striatum that causally and selectively controls excessive cocaine self-administration. This research suggests that homeostatic interactions between methyl CpG binding protein 2 (MeCP2[1]) and microRNA (miR[1])-212 in the dorsal striatum may be important in regulating vulnerability to cocaine addiction (Hollander et al. 2010; Im et al. 2010).

The research and findings we describe indicate that the field may be closer than ever to "cracking the code of addiction" (Hollander et al. 2010; Im et al. 2010; Welberg 2010).

Making Animals Addicted to Cocaine

There are many approaches to modeling both cocaine addiction as a clinical disorder and nondisordered forms of cocaine use in rats (Ahmed and Koob 1998; Belin et al. 2008; Deroche-Gamonet et al. 2004; Roberts et al. 2007). We focus on a robust modeling strategy that consists of comparing and contrasting rats with a history of extended versus limited access to cocaine for self-administration (Ahmed 2011; Ahmed and Koob 1998).

In a typical experiment, at least two matched groups of rats are allowed to self-administer cocaine intravenously for several days or weeks. The control rats have access to cocaine during only 1 hour per day and the experimental group has access for 6 or more hours per day (Ahmed and Koob 1998) (Figure 1). The original rationale of this approach was based on the assumption that extended drug use plays an etiological, though not necessarily exclusive, role in triggering the transition to cocaine addiction in humans (Ahmed and Koob 1998; Ahmed et al. 2002; Kenny et al. 2006; Koob and Le Moal 1997). Specifically, it was hypothesized that addiction-causing neuropathological processes would be set in motion only when drug exposure through self-administration (not necessarily passive exposure) increased above a certain critical level; below this level there would be no drug-induced neuropathological changes and drug use would remain under control in the majority of drug-exposed individuals. As we discuss below, this hypothesis has been confirmed by numerous findings showing that rats with a history of extended access to cocaine for self-administration develop behavioral and neurobiological alterations that are not seen in control rats with limited access.

Escalation to Heavy Cocaine Self-Administration

Escalation of drug use, a hallmark stage in the transition to addiction (Ahmed 2011), was one of the first behavioral features of addiction documented in rats with extended access to cocaine but not in control rats with limited access to the drug (Ahmed and Koob 1998) (Figure 1). Specifically, rats with extended access gradually increased intake (both total and hourly) whereas among rats with limited access intake remained remarkably stable, even after several months (Ahmed and Koob 1999).

Results show that the large majority (~70%) of individual animals with a history of extended access to cocaine for self-administration demonstrate escalation of cocaine self-administration, in contrast to a small subset of control rats (~12%) (Ahmed 2005). This outcome strongly suggests that repeated cocaine self-administration alone is necessary but not sufficient to cause escalation of cocaine use in most individuals. A certain critical level of drug self-exposure seems to be necessary to induce this behavioral feature of addiction. And indeed, rats allowed to choose the dose per injection progressively selected higher doses during extended access to cocaine for self-administration (Picetti et al. 2010).

Numerous studies have demonstrated the differential effect of drug access on cocaine self-administration (i.e.,

[1]Abbreviations that appear ≥3x throughout this article: BDNF, brain-derived neurotrophic factor; D-R, dose-response; MeCP2, methyl CpG binding protein 2; miR, miRNA, microRNA

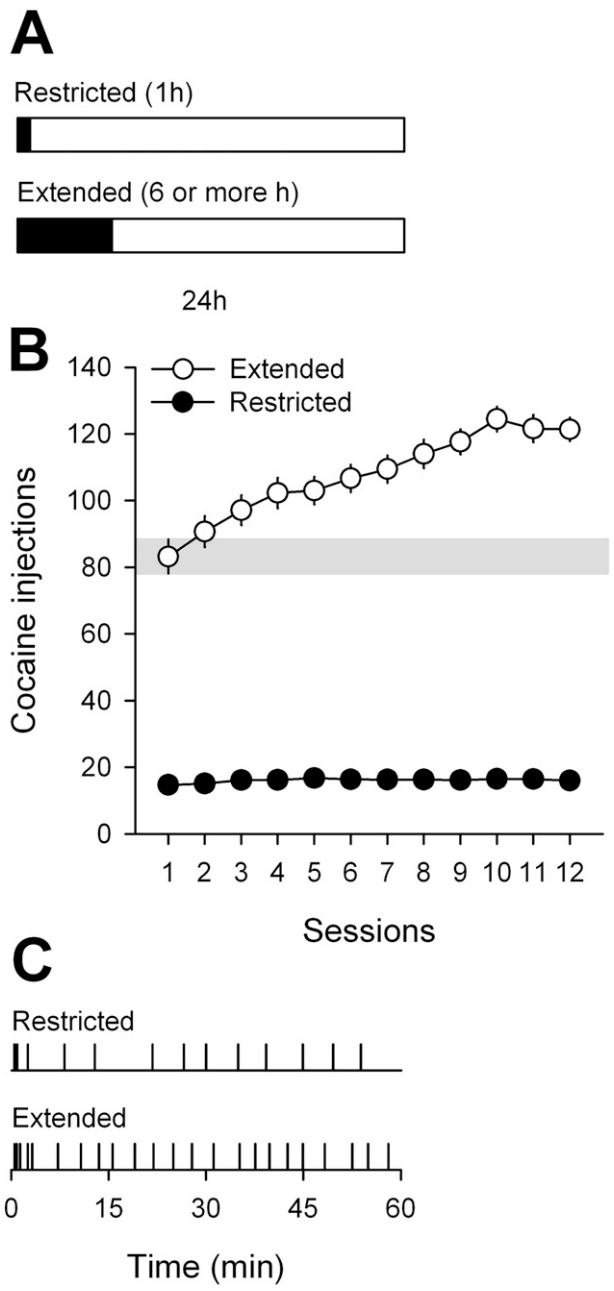

Figure 1 Effects of restricted versus extended drug access on cocaine self-administration over time. **(A)** Experimental design. After acquisition of cocaine self-administration under a fixed-ratio 1 schedule of reinforcement, rats were assigned to at least two drug intake-matched groups. Control rats (n = 30) had restricted access to cocaine for 1 hour per day and the experimental group (n = 28) had extended access for 6 or more hours per day. **(B)** Escalation of cocaine intake in rats with extended access. Data represent the mean number (± SEM) of cocaine injections (0.25 mg, administered intravenously) per session. The horizontal grey bar indicates the mean number (± SEM) of drug injections during the first day. **(C)** First-hour distribution of cocaine injections (upward ticks) by two representative individual rats, one with restricted and the other with extended drug access. Escalation of cocaine intake is largely due to acceleration in the rate of cocaine self-administration. Adapted and modified from Ahmed (2005, 2011). SEM, standard error of the mean

stability of drug use vs. escalation) (Allen et al. 2007a,b; Anker et al. 2009; Aujla et al. 2008; Ben-Shahar et al. 2004, 2006, 2008, 2009; Briand et al. 2008a,b,c; Dalley et al. 2007; Ferrario et al. 2005; Ferrario and Robinson 2007; Gipson et al. 2011; Hansen and Mark 2007; Hao et al. 2010; Hollander et al. 2010; Jin et al. 2010; Kenny et al. 2005; Madayag et al. 2007; Mantsch et al. 2004, 2008a,b; Oleson and Roberts 2009; Perry et al. 2006; Quadros and Miczek 2009; Wakabayashi et al. 2010; Wee et al. 2007a, 2008).

Studies have also reported this effect with methamphetamine (Kitamura et al. 2006; Mandyam et al. 2007; Schwendt et al. 2009; Wee et al. 2007b), heroin (Ahmed et al. 2000; Kenny et al. 2006; Lenoir et al. 2007; Lenoir and Ahmed 2008), and methylphenidate, a dopamine reuptake blocker used orally in the treatment of attention deficit hyperactivity disorder (Marusich et al. 2010). The only remarkable exception is nicotine, for reasons that have not been entirely elucidated (Kenny and Markou 2006; Paterson and Markou 2004) but could be related to the aversive effects of high doses of nicotine (Fowler et al. 2011).

Enhanced Motivation for Cocaine

Rats with extended access to cocaine for self-administration show increased motivation for the drug compared to controls with limited access. Researchers first inferred this increase in motivation from a postescalation upward shift in the peak of the dose-effect function for cocaine self-administration (Ahmed and Koob 1998; Allen et al. 2007a; Mantsch et al. 2004; Roth and Carroll 2004; Wee et al. 2007a), indicating that rats work harder to maintain the same drug effect (Ahmed et al. 2005; Christensen et al. 2008).

Direct evidence for increased drug motivation after extended cocaine use was obtained using the progressive ratio (PR) procedure (Hodos 1961; Richardson and Roberts 1996). Paterson and Markou (2003) reported that rats with a history of extended access to cocaine maintain a higher breakpoint (the maximum effort that rats accept before they stop responding for the drug) than controls, regardless of the dose available. Other investigators confirmed this observation (Allen et al. 2007b; Hao et al. 2010; Larson et al. 2007; Orio et al. 2009; Wee et al. 2008, 2009) and extended it to other drugs of abuse, including methamphetamine (Wee et al. 2007b) and heroin (Lenoir and Ahmed 2008). However, not all researchers have found an increase in breakpoint after extended cocaine use (Li et al. 1994; Liu et al. 2005; Oleson and Roberts 2009; Quadros and Miczek 2009).

Additional evidence for a postescalation increase in motivation for cocaine self-administration emerged from studies using the operant runway procedure, in which rats with a history of extended cocaine use ran faster than controls to reach a goal box where they received cocaine (Ben-Shahar et al. 2008).

Finally, using a conditioned emotional suppression procedure, Vanderschuren and Everitt (2004) found that after extended drug use rats were more likely to continue to seek cocaine despite the presence of a danger signal that normally suppresses operant behavior. Similarly, we recently found that after punishment by footshock rats with extended access to cocaine resumed drug self-administration more rapidly than controls, which refrained from self-administering cocaine for at least 3 consecutive days (Ahmed 2011).

Taken together, these findings indicate that rats with a history of extended access to cocaine for self-administration are more likely to accept a greater cost, in terms of either effort or negative consequences, to continue to seek and/or obtain cocaine, suggesting an increased motivation for the drug.

Difficulty of Abstaining from Cocaine Seeking

Difficulty of abstaining from drug seeking, another hallmark feature of addiction, can be assessed in laboratory animals by measuring their persistent drug seeking when the drug is no longer available, a behavioral pattern that illustrates resistance to extinction (Ahmed et al. 2000).

The first evidence for resistance to extinction was obtained in rats with a history of extended access to heroin self-administration (Ahmed et al. 2000; Chen et al. 2006; Doherty et al. 2009; Lenoir and Ahmed 2007, 2008); in two of these studies, resistance was present during several days (Ahmed et al. 2000; Doherty et al. 2009). In addition, the degree of resistance to extinction increased with the length of withdrawal from extended heroin self-administration, suggesting an incubation effect (Zhou et al. 2009).

Surprisingly, however, a number of studies have reported no resistance to extinction after extended access to either cocaine (Allen et al. 2007a; Jin et al. 2010; Kippin et al. 2006; Knackstedt and Kalivas 2007; Madayag et al. 2010; Mantsch et al. 2004, 2008b; Sorge and Stewart 2005) or methamphetamine for self-administration (Rogers et al. 2008; Schwendt et al. 2009). This lack of evidence for resistance to extinction may be due to the relatively short period of drug withdrawal before extinction of cocaine seeking (24–72 hours, compared to several days with heroin), presumably preventing a possible incubation effect (Grimm et al. 2001). In support of this hypothesis, when the withdrawal interval was longer (3 weeks), rats with extended cocaine use exhibited more drug-seeking responses during extinction than controls (Ferrario et al. 2005).

Alternatively, the increase in cocaine seeking after prolonged cessation of use could reflect the dissipation of some early withdrawal effects that interfered with drug seeking (e.g., general suppression of behavior and/or decreased hedonic state).

In summary, extended drug use is associated with greater difficulty abstaining from drug seeking. However, in the case of cocaine self-administration, the expression of this behavioral feature seems to require a relatively long incubation period. More research is clearly needed to test this hypothesis.

Increased Sensitivity to Drug- and Stress-Primed Craving-like Behavior

Drug-induced craving is not a current diagnostic criterion of addiction,[2] but it represents a selective feature of addiction as it is not seen in nondependent drug users (Jaffe et al. 1989; Volkow et al. 2005).

Craving-like behavior can be modeled in the laboratory by reinstatement of drug seeking after extinction (Epstein and Preston 2003). Briefly, this model involves extinction of an animal's response for the drug (by discontinuing drug delivery) and then reinstatement through exposure to a priming dose of the drug, a conditioned stimulus, or a stressor. During reinstatement testing responses are unrewarded, as during extinction, and thus reflect genuine drug-seeking behavior. Using this model, Mantsch and colleagues (2004) showed that a history of extended (but not limited) access to cocaine for self-administration was associated with an increase in cocaine-primed reinstatement of drug seeking. Other researchers have reproduced this finding (Ahmed and Cador 2006; Kippin et al. 2006; Knackstedt and Kalivas 2007; Mantsch et al. 2008b) and extended it to other drugs of abuse (heroin: Lenoir and Ahmed 2007; methamphetamine: Rogers et al. 2008; Schwendt et al. 2009).

In a study involving a within-session extinction procedure, about 80% of rats with extended access to cocaine were sensitive to intravenous cocaine-primed reinstatement of drug seeking compared to about 20% of controls (Ahmed and Cador 2006). Sensitivity to stress-primed reinstatement is also greater after a history of extended access to cocaine for self-administration (Mantsch et al. 2008a), an effect that confirms previous research with heroin self-administration (Ahmed et al. 2000). Whether sensitivity to cue-primed reinstatement changes after a history of extended access to cocaine or other stimulant drugs is less clear, however. Some studies report no change (Doherty et al. 2009; Rogers et al. 2008; Schwendt et al. 2009; Zhou et al. 2009) whereas others report a significant increase in sensitivity (Jin et al. 2010; Kippin et al. 2006).

Selective Deficits in Neurocognitive Functions

Chronic drug users addicted to cocaine present a variety of neurocognitive deficits that affect a range of higher-order functions, from attention to memory to complex decision making (Bechara 2005; Chambers et al. 2009; Garavan and Stout 2005; Goldstein et al. 2009; Paulus 2007; Robbins et al. 2008). Whether and how such deficits are a cause or consequence of drug addiction is not clear (Setlow et al. 2009).

Recent research in animals has begun to document similar deficits after extended cocaine use. Using a delayed nonmatching to sample task in a T-maze, George and colleagues (2008) observed a dramatic decrease in working memory in

[2]A craving criterion will probably be included in the next revision of the American Psychiatric Association Diagnostic and Statistical Manual of Mental Disorders (Miller and Holden 2010).

rats after extended cocaine self-administration (whereas controls with limited access to cocaine were cognitively indistinguishable from drug-naïve rats). Similarly, Briand and colleagues (2008c) have shown that rats with extended cocaine use develop a selective deficit in recognition memory that was again not present in controls. This selective deficit has also been seen in rats after extended access to methamphetamine self-administration (Rogers et al. 2008).

Prolonged cocaine self-administration can also cause transient alterations in visual attention in rats (Dalley et al. 2005). Surprisingly, however, extended access to cocaine has been shown to reduce motor impulsivity in high-impulsive rats, a paradoxical effect that is poorly understood (Dalley et al. 2007) but that could reflect a self-medication effect.

To sum up, in addition to inducing escalation of cocaine use and other addiction-like behavioral changes, animals' extended access to cocaine for self-administration can cause a variety of selective cognitive deficits that may impair their ability to regulate drug self-administration (George et al. 2008).

Compulsion and Loss of Self-Control

It is clear that after extended (but not after limited) access to cocaine for self-administration, rats develop a number of behavioral changes consistent with some symptoms of addiction: they are more likely to escalate cocaine consumption; they work harder and take more risks to seek and to obtain the drug; and they are more responsive to drug- and stress-primed craving-like behavior. But whether these changes also reflect a genuine loss of control over cocaine self-administration, which is essential to the definition of addiction as a brain disorder, is much less clear.

In all studies that have explored the behavioral effects of extended drug use, rats had no choice other than drug use. Arguably, without the possibility of a choice among rewarding options, it is difficult to determine whether rats take cocaine by compulsion or by default (Ahmed 2010). If the latter, cocaine intake may merely represent a normal response to lack of choice, not a disorder of compulsion. Thus Hyman and Malenka (2001, 702) noted that, "Although drug self-administration by rodents has provided important information, it is difficult to argue that it truly models compulsion, when the alternative to self-administration is solitude in a shoebox cage."

When rats face a choice between cocaine and an alternative nondrug reward (e.g., sweetened water), most of them abstain from drug use to consume the alternative option, despite maximal drug dose and even after a long history of extended access to cocaine for self-administration (Cantin et al. 2010; Lenoir et al. 2007). No matter how severe their past cocaine intake, only a minority of rats (fewer than 15% at the highest degree of severity) continued to take cocaine despite the opportunity to engage in an alternative behavior—even when they were hungry and offered a nondrug alternative that could relieve their vital need of calories (Cantin et al. 2010). Work is under way to address this important disparity, which may indicate that most rats, and probably most humans, are resistant to cocaine addiction.

The persistence of cocaine preference in the face of a high-stakes choice strongly suggests loss of control and compulsive cocaine use. Thus, in some rats extended access to cocaine for self-administration is associated with a genuine loss of control over intake. These rats may represent the most advanced and severe stage in the transition to cocaine addiction (Ahmed 2010; Cantin et al. 2010). Further research is needed to better assess the generalizability of this interpretation across a range of choices and settings.

Molecular Mechanisms That Regulate Vulnerability to Cocaine Addiction

We have described studies demonstrating that extended access to cocaine for self-administration triggers a multitude of complex behavioral alterations that can culminate in the development of what appears to be compulsive cocaine intake in rats. Long-lasting modifications in neuronal structure and function are thought to underlie the development of this compulsive behavior, with cocaine addiction commonly viewed as a disorder of neuroplasticity (Kalivas and O'Brien 2008; McClung and Nestler 2008).

Considering the complexity of neuroadaptive responses triggered by excessive cocaine consumption, it is likely that highly synchronized programs of gene regulation are involved. Recent findings suggest that molecular interactions in brain reward circuits between factors that coordinate gene expression and gene translation may play a key role in influencing susceptibility to compulsive cocaine use.

MicroRNAs

MicroRNAs (miRNAs[1]) are a class of small nonprotein-coding RNAs, approximately 21 to 23 nucleotides in length (Lai 2003, 2005; Lim et al. 2003), first discovered in 1993 by Victor Ambros and colleagues when they demonstrated that *lin-4* encodes a microRNA that regulates messenger RNA (mRNA) translation during *Caenorhabditis elegans* development (Lee et al. 1993).

MicroRNAs regulate gene expression at the posttranscriptional level by binding to complementary sequences (miRNA response elements) in the 3′ untranslated region (UTR) of target mRNA transcripts. Binding of miRNAs to their target transcripts can facilitate transcript degradation and/or inhibit translation (Bartel 2004). Because each miRNA may have sequence homology to many hundreds or even thousands of gene transcripts, it has the potential to be highly pleiotropic and to exert control over the expression of entire networks of related genes (Schratt et al. 2006; Wayman et al. 2008).

Neuronal cell lines express large numbers of miRNAs, many of which are associated with polyribosomes, support-

ing the notion that miRNAs regulate translational processes in neurons (Lagos-Quintana et al. 2002). Consistent with this interpretation, microarray analyses have demonstrated abundant miRNA expression, and even enrichment, throughout the mammalian brain (Miska et al. 2004; Sempere et al. 2004). Large-scale cloning and sequencing efforts have confirmed the presence of miRNAs in the adult mammalian brain (Landgraf et al. 2007), and in many cases have highlighted spatially restricted miRNAs localized to particular brain regions or cell types (Landgraf et al. 2007). Because of their ability to coordinate the expression of whole networks of related genes responsible for brain structure and function (Schratt et al. 2006; Wayman et al. 2008), miRNAs may play important roles in complex neuropsychiatric disorders, including drug addiction (Perkins et al. 2005; Rogaev 2005).

A recent series of experiments investigated the role of miRNAs in regulating escalation of cocaine intake in rats with extended daily access to the drug. Hollander and colleagues (2010) hypothesized that miRNAs whose expressions are selectively disrupted in rats with extended access to cocaine may be the most likely to influence the development of compulsive drug intake. In these experiments, alterations in miRNAs were investigated in the dorsal striatum, a key brain region that is altered in cocaine-addicted humans (Everitt and Robbins 2005; Volkow et al. 2004). Profiling revealed that expression of miR-212 was upregulated approximately 1.75-fold in the dorsal striatum of rats that had escalated their cocaine intake after extended drug access, compared to a cocaine-naïve control group (Hollander et al. 2010). In contrast, striatal miR-212 expression was not significantly elevated in rats with either limited access or extended but passive exposure to cocaine (i.e., yoked controls) (Hollander et al. 2010). Thus the upregulated miR-212 expression in the dorsal striatum of rats with escalating cocaine use appears to be related to active overconsumption of the drug, not to passive drug exposure or other behavioral actions of cocaine (e.g., chronic locomotor activation).

To investigate the functional relevance of miR-212 in regulating escalation of cocaine intake, Hollander and colleagues (2010) used a lentiviral vector to overexpress miR-212 in the dorsal striatum of rats before they self-administered cocaine under restricted access conditions. They found that the overexpression neither altered operant performance nor influenced the animals' cocaine intake. However, it profoundly altered self-administration behavior in rats with extended access to the drug: animals treated with a control lentiviral vector escalated their intake of cocaine whereas those with striatal miR-212 overexpression not only did not increase their intake but instead progressively decreased it (Hollander et al. 2010). This decrease is the opposite of the gradually escalating intake typical of rats with extended access. As such, these findings support a key role for miR-212 in regulating the vulnerability to develop compulsive cocaine intake after extended access to the drug.

Two opposing explanations might account for the effects of miR-212 on cocaine intake escalation. First, the motivational properties of cocaine may progressively decrease in rats with striatal miR-212 overexpression such that they consume less of the drug over time. Or, conversely, the hedonic impact of cocaine may progressively increase such that the rats require less and less of the drug to achieve the same level of drug reward.

To distinguish between these possibilities, the cocaine dose-response (D-R[1]) curve was characterized in control and miR-212–overexpressing rats. Left- or rightward shifts in the D-R curve are interpreted as sensitization or tolerance, respectively, to the reinforcing effects of cocaine (Ahmed and Koob 1998; Altman et al. 1996), and up- or downward shifts represent increases or decreases, respectively, in the motivation to consume the drug (Ahmed and Koob 1998; Piazza et al. 2000).

Cocaine-escalated rats typically display an upward shift in the cocaine D-R curve compared to nonescalated rats, but this upward shift was completely blocked in rats in which miR-212 was overexpressed in the dorsal striatum (Hollander et al. 2010). In fact, the curve even shifted downward in miR-212–overexpressing rats with extended access to the drug compared to those with restricted access.

As miR-212 expression is increased by ~1.75-fold in the striatum of cocaine-escalated rats, the effects described above may appear somewhat counterintuitive. A parsimonious explanation is that miR-212 upregulation represents a homeostatic response in brain reward systems to counter the escalation of cocaine intake (i.e., miR-212 is a protective factor against addiction). This would explain why ectopically overexpressing miR-212 past physiological boundaries (i.e., 6-fold upregulation) dramatically amplifies its protective action to the point of decreasing intake. Consistent with this interpretation, blockade of endogenously expressed miR-212 (through intrastriatal infusion of an antisense oligonucleotide against the miRNA) selectively increased the vulnerability to extended cocaine exposure, thereby accelerating escalation of cocaine intake (Hollander et al. 2010). Thus cocaine-induced increase in striatal miR-212 signaling represents an adaptive response in brain reward circuitries that counters the drug's addictive properties.

Taken together, these findings reveal a key role for miR-212 in regulating vulnerability to the development of compulsive cocaine intake. These data are also consistent with the notion that neuroadaptive changes occur in the brain as a response to different drug access conditions, and suggest that a mechanistic understanding of compulsive cocaine consumption will likely require the study of differences in neurobiology between rats with extended versus restricted access to the drug.

Methyl CpG Binding Protein 2 (MeCP2)

As described above, the expression of miR-212 is higher in the dorsal striatum of rats that escalated their cocaine intake and seems to protect against the development of compulsive cocaine use. MicroRNAs exert their effects through negative regulation of target gene transcripts, raising the question of

which target mRNA transcripts mediate the effects of miR-212 on escalation of cocaine intake. A recent series of experiments showed that miR-212 influences cocaine-taking behavior in part through repressive action on the transcriptional repressor MeCP2.

MeCP2 is emerging as a key regulator of many basic aspects of neuronal plasticity in postmitotic neurons in the adult brain (Nelson et al. 2006). It binds to methylated DNA to recruit histone deacetylases and other transcriptional repressors to "silence" target genes (Nan et al. 1998). Loss-of-function mutations in the MeCP2 gene cause Rett syndrome (RTT) (Amir et al. 1999; Van Esch et al. 2005), a neurodevelopmental disorder associated with severe mental retardation and characterized by compulsive behaviors (Temudo et al. 2008) related to dysfunction in dorsal striatal activity (Dunn 2001; Temudo et al. 2008). These effects suggest that MeCP2 may play a general role in compulsion-related striatal plasticity.

MeCP2 expression in the dorsal striatum is higher in rats after repeated cocaine exposure (Cassel et al. 2006), whereas levels were unaltered in "nonescalated" rats with restricted daily access to cocaine for self-administration (Im et al. 2010). These findings suggest that MeCP2 could play a role in compulsive-like cocaine use in rats with extended access to cocaine.

There is a miRNA response element (binding site) for miR-212 in the 3′ UTR of the brain-enriched form of the MeCP2 gene transcript, and miR-212 decreases MeCP2 levels in human gastric carcinoma cell lines (Wada et al. 2010). The closely related miR-132, which shares sequence homology with miR-212, represses MeCP2 in cultured mouse cortical neurons (Klein et al. 2007). These observations indicate that interactions between miR-212 and MeCP2 may influence a subject's likelihood of escalating cocaine self-administration after extended access to the drug (Im et al. 2010).

Knockdown of MeCP2 in the dorsal striatum, achieved by lentivirus-mediated delivery of an interfering short hairpin RNA, profoundly affected cocaine-taking behavior in rats with extended access to the drug (it had no effect on those with restricted access): it not only blocked cocaine intake escalation but actually induced a progressive decline in intake (Im et al. 2010). This gradual decrease in consumption is again the opposite of the gradually escalating intake typical of rats with extended access to the drug and mimics remarkably the effects of striatal overexpression of miR-212 on cocaine intake.

These findings are consistent with the idea that miR-212 may influence cocaine-taking behavior through regulation of MeCP2 levels in the dorsal striatum; specifically, as miR-212 levels increase in response to cocaine, MeCP2 expression declines, as does the motivation to consume cocaine. To test this idea, the effects of miR-212 on the expression of MeCP2 were investigated both in vitro and in vivo. Results showed that rat PC12 cells express the brain-enriched form of MeCP2 transcripts and that miR-212 overexpression reduces MeCP2 expression in these cells (Im et al. 2010). Conversely, inhibition of miR-212 signaling in PC12, achieved using an antisense oligonucleotide against miR-212, increases MeCP2 expression (Im et al. 2010). Overexpression of miR-212 in the dorsal striatum (using a lentiviral vector) led to lower MeCP2 levels in rats with either limited or extended access to the drug, but the reduction was much greater in the latter (Im et al. 2010).

These findings demonstrate that miR-212 inhibits MeCP2 expression and suggest that it may selectively decrease cocaine-taking behavior in rats with extended drug access through its repressive effect on striatal MeCP2 expression. In light of these results, which downstream signaling cascades might miR-212–MeCP2 interactions affect to influence cocaine-taking behavior?

Brain-Derived Neurotrophic Factor (BDNF)

The findings described above suggest that miR-212 controls vulnerability to compulsive cocaine taking through regulatory action on MeCP2, the levels of which closely correlate with those of brain-derived neurotrophic factor (BDNF) (Chang et al. 2006), although the precise mechanism of this interaction is unclear (Abuhatzira et al. 2007; Chahrour et al. 2008). MeCP2 overexpression increases BDNF expression in cultured mouse cortical neurons (Klein et al. 2007), whereas BDNF levels are lower in the brains of *MeCP2* mutant mice (Chang et al. 2006). Moreover, restoring brain levels of BDNF in *MeCP2* mutant mice can attenuate many of their RTT-like deficits (Kondo et al. 2008; Larimore et al. 2009).

More importantly, BDNF regulates many of the actions of cocaine. For example, infusion of BDNF into the nucleus accumbens (NAc) increases the sensitivity of rats to the psychomotor stimulant effects of cocaine (Horger et al. 1999) and induces a long-lasting increase in cocaine self-administration (Graham et al. 2007). Conversely, disruption of the *BDNF* gene in the nucleus accumbens decreases cocaine self-administration in mice (Graham et al. 2009). BDNF levels also progressively increase in midbrain dopamine and amygdala neurons after cessation of cocaine self-administration in rats and may underlie the "incubation of craving" that occurs during prolonged abstinence (Grimm et al. 2003; Lu et al. 2005).

Research has also shown that BDNF levels were selectively upregulated in the dorsal striatum of rats with extended access to cocaine and that lentivirus-mediated overexpression of BDNF in the dorsal striatum triggered an apparent loss of control over cocaine intake in these rats (Im et al. 2010). After about 2 weeks of extended access to the drug, the BDNF-overexpressing rats consumed approximately 50% more cocaine than rats treated with the control lentiviral vector (Im et al. 2010). And as their cocaine intake rapidly escalated, the BDNF-overexpressing rats showed obvious signs of deterioration in well-being—marked weight loss, agitation, and repetitive face scratching resulting in bleeding and injury (Im et al. 2010)—all of which are comparable to those of humans heavily addicted to cocaine.

In addition, BDNF-overexpressing rats showed an upward shift in the cocaine D-R curve relative to lentivirus controls with extended drug access (Im et al. 2010), whereas the curve did not differ in BDNF-overexpressing rats with restricted access. In contrast, disruption of BDNF transmission in the dorsal striatum (achieved using an anti-BDNF neutralizing antibody known to reduce BDNF transmission in rat brain; Oo et al. 2009) selectively decreased cocaine intake in the rats with extended access to the drug (Im et al. 2010). Endogenous BDNF transmission in the dorsal striatum thus selectively regulates escalation of cocaine intake under extended access conditions.

These findings reinforce the notion that neuroadaptive responses to cocaine differ markedly between rats with restricted versus extended access to cocaine, and that entirely different brain circuitries may regulate cocaine-taking behaviors in these rats.

Importantly, striatal MeCP2 knockdown reduces BDNF levels (Im et al. 2010), consistent with the positive correlation in expression between these two factors (Abuhatzira et al. 2007; Chahrour et al. 2008; Chang et al. 2006). Moreover, striatal BDNF levels were only slightly reduced by miR-212 overexpression in rats with limited access to cocaine but were almost completely ablated in those with extended access (Im et al. 2010). These differential effects closely mirror the differential action of striatal miR-212 overexpression on striatal MeCP2 levels as a function of cocaine access. The reasons for the more marked reduction in MeCP2 and BDNF levels by striatal miR-212 overexpression in rats with extended access to cocaine are unclear, but could reflect differential interactions between these molecular effectors as a function of the duration of cocaine exposure (Im et al. 2010).

Conclusions and Future Directions

The studies we have described demonstrate that miR-212 is upregulated in the dorsal striatum of rats that have escalated their cocaine intake. Upregulated miR-212 levels function as a powerful protective mechanism that limits escalation of cocaine intake, as experimentally induced increases or decreases in striatal miR-212 signaling in rats with extended drug access selectively reverse or accelerate the escalation process, respectively.

The inhibitory effects of miR-212 on excessive cocaine intake are related to an inhibitory effect on striatal MeCP2 expression and a concomitant inhibitory influence on striatal BDNF expression. As such, miR-212 may be conceptualized as a regulatory "brake" on escalation of cocaine intake. Inherited or acquired variation in the function of this molecular brake is thus likely to contribute to individual differences in vulnerability to cocaine addiction. For instance, some rats (30%) fail to show escalation of cocaine self-administration despite extended access. It is possible that this resistance to escalation results, at least partly, from preexisting high levels of striatal miR-212 signaling or other molecular changes downstream from miR-212. Conversely, few rats (12%) show escalation of cocaine intake even with limited access. The increased vulnerability to escalation may reflect preexisting disruptions in striatal miR-212 signaling.

Future research is necessary to determine the contribution of miR-212 in other complex behavioral adaptations to excessive cocaine intake that can influence the development of compulsive cocaine consumption. In particular, it will be important to determine whether the MiR-212 signaling cascade can modulate (1) drug- and stress-primed craving-like behavior and (2) the emergence of cognitive deficits that occur in rats after extended access to cocaine. Another important focus for future research will be to determine the general role of miR-212 in regulating addiction vulnerability to other drugs of abuse.

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