In the depths of the Depression, in a Manhattan alcoholism clinic, a ruined Wall Street speculator named Bill Wilson had a vision. His room suddenly blazed “with an indescribably white light” and he experienced euphoria and a godlike “presence”, followed by a “great peace”. Like St Paul after his experience on the road to Damascus, Wilson soon turned away from his old, inebriated life and became an evangelist — preaching a radical, spiritual cure for alcoholism.

That cure grew into the modern addiction rehabilitation industry, which even today is dominated by Wilson’s Alcoholics Anonymous (AA) paradigm and its ‘twelve-step’ approach to recovery. Perhaps unsurprisingly, given its spiritual origins, this approach has had an uneasy relationship with the evidence-based culture of medical research. Both perceive addiction as a chronic disease; but whereas scientists seek rationally targeted interventions to blunt drug cravings, AA and related programmes tend to feature group therapy, tearful confessions and the call to “surrender to a higher power”.

In the past few years, however, these two cultures have been finding common ground. Neuroscientists have begun to recognize that some of the most important brain systems impaired in addiction are those in the prefrontal cortex that regulate social cognition, self-monitoring, moral behaviour and other processes that the AA-type approach seems to target. “A lot of the treatment programmes out there are targeting these systems without necessarily knowing that they are doing it,” says Nora Volkow, director of the National Institute on Drug Abuse in Bethesda, Maryland.

Researchers are now searching for ways to boost these prefrontal systems even further — not to remove the need for twelve-step and other behaviourally oriented treatment programmes, but to enable people with addictions to get more out of them. “It completely changes the way that we look at medications,” Volkow says.

Until recently, addiction researchers focused almost entirely on ‘limbic’ circuits in the brain that mediate fear and desire. These dopamine-fuelled networks are effectively hijacked by addictive drugs and behaviours so that the person ends up wanting, and compulsively seeking, little else but the next fix. Drugs such as methadone and naltrexone can blunt the activity of these circuits, but they are not a cure.

Impulse management

While doing neuroimaging studies at the Brookhaven National Laboratory in Upton, New York, in the 1990s, Volkow was one of the first researchers to suggest that abnormalities in the prefrontal cortices of drug users might weaken the systems that normally counteract drug cravings. Since then, the prefrontal regions and their links to the limbic system have garnered more and more attention, and researchers are now attempting “a very extensive evaluation of how the different areas in the prefrontal cortex participate in the process of drug addiction”, Volkow says.

The prefrontal cortex — the most recently
evolved set of structures in the brain and the one that most clearly differentiates humans from other species — is the headquarters for the circuits that help shape feelings and behaviour according to long-term goals, moral strictures and social cues. These systems are extensively wired into limbic regions, and are often portrayed as a ‘braking’ system to resist impulsive behaviour. The slow development of prefrontal structures after birth tracks the maturation of children into adults, and people whose prefrontal areas are damaged by trauma or stroke, for example, seem to have lost some control of the brakes and are apt to be childishly impulsive and uninhibited in their behaviour.

With tools such as psychological tests and brain imaging, researchers have been finding similar braking problems associated with drug use and are starting to tease apart the mechanisms involved. Some have shown that people with drug addictions are poor at monitoring their own behaviour, making appropriate decisions and inhibiting impulses — and these behavioural findings have been matched to functional magnetic resonance imaging (fMRI) data that show reduced activity in the corresponding prefrontal areas. Animal studies have supported the human ones by showing, for example, that monkeys given cocaine swiftly develop prefrontal impairments. And other researchers have found that stress, which frequently triggers drug use and relapse in people with addiction, seems to do so at least in part by shutting down prefrontal functions. “We’re really starting to understand the molecular basis of why this cortex falls apart with drugs of abuse, and during stress, and how those two interact,” says Amy Arnsten at Yale University School of Medicine in New Haven, Connecticut.

If the cortex falls apart with drug abuse, then it may be impossible to recover from an addiction without putting it back together. In unpublished studies, Hugh Garavan and his colleagues at Trinity College, Dublin, have found that cocaine users and tobacco smokers who go through treatment and are able to stay abstinent for more than a year “seem to show hyperactivity in these prefrontal control centres” in fMRI images. Garavan says that this extra activity seems to be especially prominent during the first few weeks of abstinence, hinting at “a heavy reliance on these prefrontal centres to avoid falling off the wagon”.

The recognition that prefrontal systems might need boosting in people with addictions has helped fuel a new interest in whether AA and similar behavioural treatments are already having these kinds of effects. “It behooves us to try to understand how [twelve-step approaches] link to what we’re addressing in terms of intervention,” Volkow told the annual meeting of the Society for Neuroscience in Washington DC last November. So far, these treatment programmes have been difficult to study formally, says Martin Paulus, a psychiatrist who is researching addiction at the University of California, San Diego. “It’s very much a voluntary-based programme, with little standardization, and the whole programme thrives on anonymity.”

But much of what is known about the AA approach suggests that it aims to protect or enhance prefrontal circuits. In the protected environment of a rehab centre, drugs and other cues associated with drug taking are gone and stressful situations that suppress prefrontal activity are minimized. Volkow notes that the feeling of ceding control to a higher power is also likely to “enhance your sense of security, decreasing stress and anxiety”. Similarly, says Garavan, the confessions of bad behaviour and other “strategies that push users to become more aware of their drug-related actions presumably aim to boost their capacity for self-monitoring, which is largely a prefrontal function”.

The social environment in rehab is another factor that works in part through prefrontal systems. “Our brains have evolved to be very sensitive to social cognition and social reinforcers,” says Volkow. By putting people with drug addictions into a group with anti-drug values, “you are providing them with a very powerful reinforcer”, she says.

**Spiritual control**

And then there is religion, which has been shown to have a strong inverse association with drug addiction. Psychologist Michael McCullough, who studies religion and behaviour at the University of Miami in Florida, calls this inverse association “one of the most unsung findings in the entire literature on drug and alcohol abuse”. Both adults and children deemed religious by various measures “drink, smoke and do drugs less often”, McCullough says. “If they get into trouble with drinking and drugs and smoking, they’re more likely to be able to get away from those problems.”

McCullough suggests that when a person commits to any cultural system that regulates behaviour, the psychological effort to conform strengthens the brain systems that mediate self-monitoring and self-control. “What makes religion unique, I think, is that the code of conduct isn’t just laid down by your parents or your friends or your principal at school, but ostensibly by the individual who is superintending the Universe, so it has an extra moral force.” Some religious rituals, he says, have been shown to provoke enhanced activity in prefrontal regions. “It’s as if certain forms of prayer and meditation are pinpointing precisely those [prefrontal] areas of the brain that people rely on to control attention, to control negative emotion and resolve mental conflict.”

However the twelve-step strategies actually work on the brain, “there is now excellent documentation that those who attend AA-type programmes regularly do very well by anyone’s standard”, says Thomas McLellan, director of the Treatment Research Institute in Philadelphia, Pennsylvania. The problem, McLellan says, is that the vast majority of people who enter such programmes do not go regularly — they drop out after a few days or weeks — and are more than likely to relapse.

Anna Rose Childress, a psychiatrist at the University of Pennsylvania School of Medicine, who is researching addiction...
Medicine in Philadelphia, has encountered a similar resistance to treatment in the crack cocaine users she has studied. In her lab she uses a cognitive behavioural training technique — like “prefrontal push-ups”, she says — that tries to make these users more aware of their drug-related actions and the consequences. But her studies indicate that “most of our cocaine patients are not great at it.”

Results such as these raise what Childress and others call the “chicken or egg question” — is drug use the cause of users’ prefrontal problems, or do they have pre-existing deficits that make them susceptible to addiction? As Garavan puts it: “A lot of people might be able to enjoy drugs but there’s only a certain percentage who actually go on to become addicted. And maybe part of that is because these people lack that prefrontal-mediated control over behaviour.”

Some research already links prefrontal-related conditions such as impulsivity and attention deficit hyperactivity disorder (ADHD) to a heightened risk of later drug use. But to really start answering the chicken or egg question, says Childress, “you would need some good large-scale developmental studies for one thing; you would like to look at adolescents before they’ve ever touched drugs”.

Garavan and several dozen other European researchers are now participating in a project that aims, in part, to do just that. Known as IMAGE and begun in late 2007, the five-year, €10-million (US$14-million) project funded by the European Commission will ultimately enrol 2,000 14-year-olds and follow them through their late teens. Principal investigator Gunter Schumann, a psychiatrist at Kings College, London, says that the testing will include fMRI and structural MRI, as well as a full genome scan. He expects to start publishing findings in the next few years.

**Quenching the flame**

In the meantime, researchers are pursuing other ways to boost prefrontal systems — and medicines for ADHD seem an obvious place to start.

Attention-enhancing drugs such as methylphenidate and atomoxetine boost the activity of key receptor systems in the prefrontal cortex, in particular those for noradrenaline and dopamine. Some evidence already suggests that patients with ADHD are less likely to go on to abuse drugs if they are receiving medication for their condition. And earlier this year, a team led by Daina Economidou at the University of Cambridge, UK, reported that atomoxetine helped rats with an ADHD-like impulsivity to resist a relapse to cocaine-seeking.

The National Institute on Drug Abuse has also been supporting studies of cognitive and behavioural strategies, and Volkow says that she is particularly enthusiastic about an approach that involves “real-time fMRI feedback”. Developed by researcher and entrepreneur Christopher deCharms earlier this decade, the technique involves placing drug users in an fMRI machine and showing them a symbolic representation — a flame — of the fMRI-measured brain activity that corresponds to their cravings. The users are then asked to apply their own cognitive exercises, such as imagining their child is with them, to quench their cravings and douse the flame. After half a dozen sessions with this feedback the user will, in principle, develop cognitive circuitry that is more efficient at suppressing craving and that can then be used in ordinary life. A version of the technique, used for pain relief, has already shown some efficacy in a small clinical trial, and deCharms and his Silicon Valley start-up, Omneuron, are currently running a small trial in smokers — with plans for a follow up with some of Childress’s cocaine users.

For some people, even the most sophisticated therapies may not be enough to rescue a prefrontal cortex that has been damaged by genetics, development and perhaps decades of drug use. “It’s like somebody who has had a stroke and is paralysed,” says psychologist Antoine Bechara at the University of Southern California, “and you tell them, well, you should walk, you should exercise. But the part of the brain that allows them to do that is not there and they just cannot do it.”

To Bechara, a more efficient approach would be to protect and strengthen these critical brain regions as they are developing. As an example, he cites preliminary data from a study in China. “There are children who grow up whose parents make all the decisions for them, and others who are encouraged to make decisions and are rewarded or punished for their bad decisions,” he says. "The latter children grow up to show better performance on measures of decision making, and there is even a hint of evidence from fMRI that the kids with that latter kind of parenting style have better prefrontal cortex function.”

Even for those beyond the influence of parenting style, researchers hope that a little lift in prefrontal efficiency could go a long way. Such a boost, says Paulus, could be "the critical piece that helps prevent the person from getting onto a very destructive pathway".

The question now is how best to give that boost. As researchers come to understand the neural mechanisms of addiction better, the twelve-step approach may give way to more secular strategies. But it seems unlikely that all behavioural approaches will soon be replaced by a pill. “I think most research would say, and I know I would say, that medicines should be used in the context of a good behavioural programme,” says Childress, “because a person is essentially trying to restructure a lot of behaviour, and the more support that you can provide for that, the better.”

**Jim Schnabel is a freelance writer based in Maryland.**

Addiction and Cognition

The brain regions and neural processes that underlie addiction overlap extensively with those that support cognitive functions, including learning, memory, and reasoning. Drug activity in these regions and processes during early stages of abuse foster strong maladaptive associations between drug use and environmental stimuli that may underlie future cravings and drug-seeking behaviors. With continued drug use, cognitive deficits ensue that exacerbate the difficulty of establishing sustained abstinence. The developing brain is particularly susceptible to the effects of drugs of abuse; prenatal, childhood, and adolescent exposures produce long-lasting changes in cognition. Patients with mental illness are at high risk for substance abuse, and the adverse impact on cognition may be particularly deleterious in combination with cognitive problems related to their mental disorders.

Drug addiction manifests clinically as compulsive drug seeking, drug use, and cravings that can persist and recur even after extended periods of abstinence. From a psychological and neurological perspective, addiction is a disorder of altered cognition. The brain regions and processes that underlie addiction overlap extensively with those that are involved in essential cognitive functions, including learning, memory, attention, reasoning, and impulse control. Drugs alter normal brain structure and function in these regions, producing cognitive shifts that promote continued drug use through maladaptive learning and hinder the acquisition of adaptive behaviors that support abstinence.

In a 2005 review, Steven Hyman stated the current neurological conception of drug abuse concisely: Characterizing addiction as a disease of “pathological learning,” he wrote, “[A]ddiction represents a pathological usurpation of the neural mechanisms of learning and memory that under normal circumstances serve to shape survival behaviors related to the pursuit of rewards and the cues that predict them.”

This article reviews current knowledge on the cognitive effects of drugs and their neurological underpinnings. These effects may be particularly disruptive when individuals are exposed to drugs during brain development, which lasts from the prenatal period through adolescence, and in individuals with mental disorders. An understanding of these issues will help substance abuse clinicians identify and respond to cognitive changes that affect patients’ responses to treatment.
A MULTISTAGE PROCESS
Recent reviews characterize addiction as a two-stage process. In the first stage, the individual's occasional drug taking becomes increasingly chronic and uncontrolled. The neurological source of these symptoms is drug-induced deregulation of the brain's reward system (Feltenstein and See, 2008). Normally, increased dopamine signaling within this system—specifically, in the ventral striatum or nucleus accumbens (NAc)—produces pleasurable feelings that orient organisms to seek and perform life-sustaining conditions and activities, such as locating supportive environments, eating, and having sex. Drugs of abuse hyperactivate this system, triggering abrupt and large increases in NAc dopamine signaling, producing intense sensations that motivate additional drug taking, and promoting the formation of maladaptive drug-stimulus associations (Feltenstein and See, 2008).

Individuals in the second stage of the addictive process present additional clinical features, including withdrawal symptoms during early abstinence, persistent vulnerability to relapse, and alterations in decisionmaking and other cognitive processes. Although modification of the dopaminergic reward system remains important at this stage, it probably is not sufficient to maintain these complex and long-lasting changes. Kalivas and Volkow (2005) summarize evidence implicating drug-induced alterations in signals carried by the neurotransmitter glutamate from the brain area that is primarily associated with judgment—the prefrontal cortex—to the NAc. Le Moal and Koob (2007) emphasize changes in brain stress circuits and negative reinforcement (i.e., effects that motivate drug taking by causing discomfort during abstinence, such as the onset of withdrawal symptoms). Thus, whereas early drug use fosters maladaptive drug-stimulus associations that contribute to drug seeking and use, later stages disrupt cognitive and other processes that are important for successful abstinence.

The full extent of drugs' impacts on cognition is not yet known, but research indicates that addicted individuals have alterations in brain regions including the striatum, prefrontal cortex, amygdala, and hippocampus (Jones and Bonci, 2005; Kalivas and Volkow, 2005; Kelley, 2004; Le Moal and Koob, 2007). These same regions underlie declarative memory—the memories that define an individual, without which it would be difficult to generate and maintain a concept of self (Cahill and McGaugh, 1998; Eichenbaum, 2000; Kelley, 2004; Setlow, 1997). Drugs’ capacity to act upon the substrates of declarative memory suggests that their impact on cognition is potentially extremely far-reaching.

COGNITIVE EFFECTS OF ACUTE DRUG ADMINISTRATION
Clinicians often observe that patients undergoing treatment for addiction become highly vulnerable to relapse when they return to contexts or environments where their addiction developed (Hyman, 2005; See, 2005). Clinical research confirms that cues associated with substance abuse elicit physiological responses and cravings for drugs (Franklin et al., 2007). Laboratory animals, too, develop powerful associations and cue-response behaviors in the presence of drug-related stimuli. For example, animals given a drug in one compartment of a double cage subsequently will gravitate to that compartment more than to the alternative compartment. This phenomenon, known as conditioned place preference, has been demonstrated in studies using nicotine, ethanol, amphetamine, methamphetamine, cocaine, morphine, cannabis, and caffeine (Bardo and Bevins, 2000).

The Formation of Drug-Stimulus Associations
The multistage model of addiction attributes addicted individuals’ strong responses to drug cues to a learning process that inculcates powerful drug-stimulus associations (e.g., Robinson and Berridge, 2000). In this view, the individual taking a drug perceives his or her present surroundings as highly significant (salient) and makes exceptionally strong mental connections between features of those surroundings and the intense pleasure of the drug. Subsequently, when he or she re-encounters those features, the powerful associations reassert themselves, consciously or subconsciously, and are experienced as prompts for drug seeking and drug taking. Consistent with this account, exposing addicted individuals to cues that they associate with substance abuse elicits, along with physiological responses and drug cravings, changes in the activity levels of brain regions involved in learning and memory (i.e., striatum, amygdala, orbitofrontal cortex, hippocampus, thalamus, and left insula) (Franklin et al., 2007; Volkow et al., 2006).

The acute effects of amphetamine, nicotine, and cocaine fit straightforwardly into this scenario. Each of these drugs has been shown to acutely enhance learning and/or attention (Del et al., 2007; Kenney and Gould, 2008; Matray, 1996). For example, the idea that smoking is a cognitive enhancer is well accepted by researchers and the general public. Numerous studies
have confirmed that laboratory animals’ cognitive processes improve immediately following administration of nicotine (Kenney and Gould, 2008). Similar findings in early studies with human smokers were not conclusive, because the study participants were smokers who had received nicotine following a period of abstinence. The observed enhancements might have reflected the reversal of withdrawal effects, rather than improvements over their normal cognitive powers. A subsequent review of the literature, however, suggests that acute nicotine enhances reaction time and attention in nicotine-naïve individuals (Swan and Lessov-Schlaggar, 2007). Cocaine produced similar effects in a study of rats that were treated with the drug and then exposed to a sensory stimulus; the animals exhibited enhanced neural activation when later re-exposed to the stimulus (Devonshire, Mayhew, and Overton, 2007).

Although all drugs of abuse foster the learning of strong drug-stimulus associations and cue-induced drug seeking, some appear to have mixed effects on other types of learning and cognition. For example, a clinical study of the acute effects of morphine and oxycodone concluded that these drugs have variable impacts on cognition: Both improved men’s recall of prose just slightly, but morphine slightly impaired both sexes’ performance on a test of working memory in which they were asked to repeat a set of digits in reverse order (Friswell et al., 2008). In another study, mice were given morphine or saline and trained to run away when a light signaled that a foot shock was impending; although the morphine–treated mice scored higher on the frequency and quickness with which they avoided shocks, the researchers attributed this to increased motor activity rather than enhanced learning (Aguilar, Miñarro, and Simón, 1998).

### The Persistence of Drug-Stimulus Associations

Recent research has sought to account for the strikingly long-lasting ability of maladaptive drug-stimulus associations to influence behavior and provoke relapse. Studies have shown that many abused substances can reshape the communication pathways between neurons (synaptic plasticity), which could contribute to both the formation and the persistence of maladaptive drug-stimulus associations.

Cocaine and nicotine can directly induce one form of synaptic plasticity, the strengthening of neural connections via a process known as long-term potentiation (LTP; see Learning in the Mind and Brain on page 8 and Table 1) (Argilli et al., 2008; Kenney and Gould, 2008). Amphetamine can enhance LTP (Delanoy, Tucci, and Gold, 1983). Marijuana activates the endocannabinoid system, resulting in inhibition in some instances and facilitation in others of both LTP and long-term depression (LTD), another form of synaptic plasticity in which connections between neurons become less responsive (Carlson, Wang, and Alger, 2002; Nugent and Kauer, 2008; Sullivan, 2000). Ethanol consistently disrupts LTP while enhancing LTD (Yin et al., 2007). Morphine inhibits LTP of neurons that exhibit inhibitory control of neural activity via the neurotransmitter gamma-aminobutyric acid (GABA) (Nugent and Kauer, 2008). Inhibition of GABA activity could lead to an overall increase in neural activity throughout the brain, which might lead to the formation of stronger associations than would normally occur, including maladaptive drug-context associations.

### TABLE 1: Drug Effects on Synaptic Plasticity

<table>
<thead>
<tr>
<th>DRUG</th>
<th>EFFECTS ON PLASTICITY</th>
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<tr>
<td>Amphetamine</td>
<td>LTP</td>
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<tr>
<td>Cocaine</td>
<td>LTP</td>
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<tr>
<td>Ethanol</td>
<td>LTP, LTD</td>
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<tr>
<td>Marijuana</td>
<td>LTP, LTD</td>
</tr>
<tr>
<td>Morphine</td>
<td>LTP (of inhibitory synapses)</td>
</tr>
<tr>
<td>Nicotine</td>
<td>LTP</td>
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LTP, long-term potentiation of synaptic efficiency; LTD, long-term depression of synaptic efficiency.

In contrast to the effects of opioids on cognition, those of alcohol are clear, though bidirectional: High doses disrupt cognitive processes (Ryback, 1971), while low doses can enhance learning (Gulick and Gould, 2007; Hernández, Valentine, and Powell, 1986).
COGNITIVE DEFICITS IN CHRONIC DRUG ABUSE

Drug abusers who progress to the second stage of addiction are subject to withdrawal when they initiate abstinence. Many drugs produce cognition-related withdrawal symptoms that may make abstinence more difficult. These include:

- **cocaine**—deficits in cognitive flexibility (Kelley et al., 2005);
- **amphetamine**—deficits in attention and impulse control (Dalley et al., 2005);
- **opioids**—deficits in cognitive flexibility (Lyvers and Yakimoff, 2003);
- **alcohol**—deficits in working memory and attention (Moriyama et al., 2006);
- **cannabis**—deficits in cognitive flexibility and attention (Pope, Gruber, and Yurgelun-Todd, 2001); and
- **nicotine**—deficits in working memory and declarative learning (Kenney and Gould, 2008).

Nicotine provides a familiar example of cognitive changes in withdrawal. In both chronic smokers and animal models of nicotine addiction, cessation of nicotine administration is associated with deficits in working memory, attention, associative learning, and serial addition and subtraction (Bell et al., 1999; Blake and Smith, 1997; Davis et al., 2005; Hughes, Keenan, and Yellin, 1989; Jacobsen et al., 2006; Mendrek et al., 2006; Raybuck and Gould, 2009; Semenova, Stolerman, and Markou, 2007). Moreover, it has been shown that the severity of decreases in cognitive performance during periods of smoking abstinence predicts relapse (Patterson et al., 2010; Rukstalis et al., 2005). Although these deficits usually dissipate with time, a dose of nicotine will rapidly ameliorate them (Davis et al., 2005)—a situation that may contribute to some relapses. Thus, chronic substance abuse can lead to cognitive deficits that are particularly pronounced during early periods of abstinence.

While the cognitive deficits associated with withdrawal from drugs are often temporary, **long-term use can also lead to lasting cognitive decline.** The nature of deficits varies with the specific drug, the environment, and the user’s genetic makeup (see Genes, Drugs, and Cognition on page 11). In general, however, they impair the ability to learn new patterns of thought and behavior that are conducive to successful response to treatment and recovery.

For example, long-term cannabis users have impaired learning, retention, and retrieval of dictated words, and both long-term and short-term users show deficits in time estimation (Solowij et al., 2002), although how long these deficits persist is not yet known. As another example, chronic amphetamine and heroin users show deficits in a range of cognitive skills, including verbal fluency, pattern recognition, planning, and the ability to shift attention from one frame of reference to another (Ornstein et al., 2000). The decisionmaking deficits resembled those observed in individuals with damage to the prefrontal cortex, suggesting that both drugs alter function in that brain area (Rogers et al., 1999).

A pair of recent studies suggests that some methamphetamine-induced cognitive losses may be partially...
LEARNING IN THE MIND AND BRAIN

A mind learns: It captures and stores information and impressions and discovers relationships between them. For the mind to learn, events must occur in the brain. Among the most compelling pieces of evidence for this idea are many cases of individuals who suffered drastic reductions of their ability to learn after incurring brain injuries. The most famous, perhaps, is Henry Molaison, who, after surgical removal of extensive brain tissue at age 27 to control his epilepsy, entirely lost his long-term declarative memory (Penfield and Milner, 1958) so that for the remaining 55 years of his life he could not call to mind anything that happened to him more than a few minutes earlier.

Neuroscience research has correlated learning with the elaboration of neural networks in the brain. Many experiments have established that, as learning takes place, selected neurons increase their levels of activity and form new connections, or strengthen established connections, with networks of other neurons. Moreover, experimental techniques that prevent neuronal activity and networking inhibit learning.

Neuroscience research with animals is elucidating how the brain constructs and maintains the neural networks that support learning. One key aspect of learning is LTP, or long-term potentiation, which describes a biochemical change that can result in a prolonged increase in neuronal sensitivity to the stimuli that evoked the change. Its discovery in the late 1960s was a landmark in the study of learning, memory, and addiction.

Once we learn to associate two ideas or sensations, the occurrence of one is likely to invoke remembrance of the other. Similarly, in LTP, a neuron that receives strong, or high-frequency, stimulation from another neuron responds by becoming more sensitive to future stimulation from the same source.

Newly learned material enters our short-term memory and may or may not subsequently become established in our long-term memory. Similarly, LTP has an early phase during which short-term physiological processes support the above-mentioned increase in neuronal sensitivity and a late phase involving more long-lasting physiological processes.

Animal studies have implicated some of the same sequences of biochemical changes (cell signaling cascades) in LTP and learning. For example, researchers showed that suppressing production of an enzyme (protein kinase A) in the hippocampi of mice prevented LTP and inhibited the animals’ ability to retain previously learned information about a maze (Abel et al., 1997).

Although LTP has not been observed in every brain region, it has been demonstrated in the nucleus accumbens, prefrontal cortex, hippocampus, and amygdala—all regions involved in both addiction and learning (Kenney and Gould, 2008; Kombian and Malenka, 1994; Maren, 2005; Otani et al., 2003).

Newly learned material remains in short-term memory for less than 6 months, chronic methamphetamine abusers scored lower than unexposed controls on tests of motor function, memory for spoken words, and other neuropsychological tasks. The deficits were associated with a comparative scarcity of dopamine transporters (proteins that regulate dopamine) and reduced cellular activity (metabolism) in the thalamus and NAc. When retested after 12 to 17 months of abstinence, the drug abusers’ motor function and verbal memory had risen to levels that approached those of the control group, and the gains correlated with a return toward normal transporter levels in the striatum and metabolic levels in the thalamus; however, other neuropsychological deficits remained, along with depressed metabolism in the NAc.

In another study, abusers of 3,4-methylenedioxy-methamphetamine (MDMA, ecstasy) continued to score relatively poorly in tests of immediate and delayed recall of spoken words even after 2.5 years of abstinence (Thomasius et al., 2006). In a study of polydrug abusers who had stated a primary preference for either cocaine or heroin, deficits in executive function—defined as changes in fluency, working memory, reasoning, response inhibition, cognitive flexibility, and decisionmaking—remained after up to 5 months of abstinence (Verdejo-García, and Pérez-García, 2007).

An important question is whether nicotine’s cognitive benefit persists as smoking shifts from sporadic to chronic. In some studies with animals, chronic nicotine administration improved cognitive capacities such as attention, but other studies found that initial improvements waned with chronic treatment (Kenney and Gould, 2008). Furthermore, several recent studies have shown that smoking and a past smoking history are associated with cognitive decline. For example, in one study with middle-aged men and women, smokers’ cognitive speed declined nearly twice as much as nonsmokers’ over 5 years; in addition, declines in smokers’ cognitive flexibility and global cognition occurred at 2.4 times and 1.7 times the respective rates of nonsmokers (Nooyens, van Gelder, and Verschuren, 2008). Recent quitters’ scores in these areas were similar to smokers’, and ex-smokers performed at levels intermediate between smokers and nonsmokers.

Similarly, in another study, smokers’ performance deteriorated more over 10 years than nonsmokers’ on tests of verbal memory and speed of visual searching; ex-smokers’ visual search speed slowed more than nonsmokers’ as well (Richards et al., 2003). Although some early studies suggested that smoking might retard the cognitive decline associated with Alzheimer’s disease (van Duijn and Hofman, 1991), followup studies failed...
to confirm this, and others correlated smoking quantity and duration with higher risk for Alzheimer’s disease (Swan and Lessov-Schlaggar, 2007).

Laboratory studies have demonstrated nicotine-related alterations in neuronal functioning that could underlie cognitive decline that persists even after prolonged abstinence. For example, rats’ self-administration of nicotine was associated with a decrease in cell adhesion molecules, a decrease in new neuron production, and an increase in cell death in the hippocampus (Abrous et al., 2002). Such changes could result in long-lasting cognitive changes that contribute to poor decision-making and addiction.

DRUGS OF ABUSE AND THE DEVELOPING BRAIN
The human brain continues to develop and consolidate important neural pathways from the prenatal period through adolescence. Throughout these years, the brain is highly malleable, and drug-induced alterations of neural plasticity may deflect the normal course of brain maturation.

Prenatal Exposures
The consequences of prenatal alcohol exposure are well-known: Fetal alcohol spectrum disorders are the leading cause of mental retardation in the United States (Centers for Disease Control and Prevention, 2009). In addition, fetal alcohol exposure increases susceptibility to later substance abuse problems (Yates et al., 1998).

Prenatal exposures to a number of other drugs have significant deleterious effects on cognition and behavior that may not rise to the level of mental retardation. In one study, 5-year-olds whose mothers had used alcohol, cocaine, and/or opiates while pregnant ranked below unexposed controls in language skills, impulse control, and visual attention. There were no significant differences between the two groups of children in intelligence, visual/manual dexterity, or sustained attention; however, both groups placed below the normative means on these measures (Pulsifer et al., 2008). Another study documented memory deficits in 10-year-old children who had been exposed prenatally to alcohol or marijuana (Richardson et al., 2002).

Clinical and laboratory research has implicated prenatal exposure to methamphetamine in both cognitive deficits and altered brain structure. For example, one study correlated shorter attention span and delayed memory with reduced volume in the putamen (-18 percent), globus pallidus (-27 to -30 percent), and hippocampus (-19 to -20 percent) among 15 children aged 3 to 16 years who were prenatally exposed to the stimulant, compared with controls (Chang et al., 2004). The drug-exposed children also exhibited poorer long-term spatial memory and visual/motor integration. Another study documented structural changes in the frontal and parietal cortex of 3- and 4-year-old children who had been exposed prenatally to methamphetamine (Cloak et al., 2009). In laboratory studies, rats that were treated with methamphetamine during pregnancy gave birth to pups that, when they reached adulthood, were slow to learn spatial relationships and exhibited spatial memory impairment (Acuff-Smith et al., 1996; Slamberová et al., 2005).

The effects of prenatal tobacco exposure are particularly concerning because so many expectant mothers smoke—by one estimate, over 10 percent in the United States (Hamilton et al., 2007). In utero exposure to tobacco byproducts has been linked to cognitive deficits in laboratory animals and human adolescents (Dwyer, Broide, and Leslie, 2008). Some studies suggest that such exposure can lower general intelligence; for example, one found a 12-point gap in full-scale IQ between exposed and unexposed middle-class adolescents (e.g., Fried, Watkinson, and Gray, 2003). In another study, the odds of having attention deficit hyperactivity disorder (ADHD) were more than three times as great for adolescents whose mothers smoked during pregnancy compared with children of nonsmoking mothers (Pauly and Slotkin, 2008).

Cognitive deficits following prenatal exposure to smoking may reflect structural brain changes. In one study, prenatally exposed adolescent smokers had greater visuospatial memory deficits in conjunction with changes in parahippocampal and hippocampal function compared with adolescent smokers not prenatally exposed (Jacobsen et al., 2006). Brain imaging of adolescent smokers and nonsmokers who were prenatally exposed to smoking has revealed reduced cortical thickness (Toro et al., 2008) and structural alterations in cortical white matter (Jacobsen et al., 2007). Furthermore, in rats, prenatal exposure to nicotine decreased memory-related neural activity in the hippocampus and resulted in deficits in active avoidance learning, with male and female prenatally exposed rats showing significantly fewer correct responses as young adults (Vaglenova et al., 2008). These deficits persisted into later adulthood among the male rats, but not the females.
Among the adverse consequences of prenatal drug exposure is a heightened risk of becoming a drug abuser in later life (Fergusson, Woodward, and Horwood, 1998). This is troubling, as it may lead to a downward spiral that manifests across generations and destroys family structures. Multiple factors could contribute to the increased risk of future substance abuse, including the effects of prenatal drug exposure on cognition. As already reviewed, the risk of developing ADHD is greatly increased in adolescents whose mothers smoked during pregnancy (Pauly and Slotkin, 2008). ADHD is often comorbid with substance abuse (Biederman et al., 2008; Molina and Pelham, 2003), suggesting a link between such changes in cognition and future drug abuse. Further work is needed to understand the mechanisms that underlie the increased risk of drug abuse associated with prenatal exposure.

Adolescent Exposure

Adolescence is a high-risk period for substance abuse. Most addicted smokers first formed the habit during adolescence (Khuder, Dayal, and Mutgi, 1999). Adolescent smoking strongly affects cognition. Adolescent smokers scored worse than age-matched nonsmokers on tests of working memory, verbal comprehension, oral arithmetic, and auditory memory (Fried, Watkinson, and Gray, 2006; Jacobsen et al., 2005). These deficits resolved upon cessation of smoking with the exceptions of working memory and arithmetic performance, which remained at comparatively low levels. In rats, nicotine exposure during adolescence was associated with visuospatial attention deficits, increased impulsivity, and increased sensitivity of medial prefrontal cortical dopamine terminals in adulthood (Coutotte et al., 2009). In addition, adolescent rats treated with nicotine had long-lasting changes in the sensitivity of the adenylyl cyclase cell signaling cascade (see Figure 1), a second messenger pathway involved in many processes, including learning and memory (Slotkin et al., 2008). These findings fit well with studies demonstrating that nicotine initially can enhance some cognitive processes, but with continued use adaptation can occur, leading to dissipation of these effects and even deficits (for review, see Kenney and Gould, 2008).

Adolescent smoking can foster cognitive decline indirectly, through the promotion of other disorders. For example, adolescent cigarette use is associated with later episodes of depression (Choi et al., 1997), a malady which in turn is associated with negative effects on cognition (Thomas and O’Brien, 2008). A laboratory investigation shed light on this relationship: Adult rats that had been exposed to nicotine during their adolescence proved less sensitive than controls to rewarding/appetitive stimuli and more responsive to stress and anxiogenic stimuli (Iñiguez et al., 2009).

Adolescent exposures to other substances of abuse, such as alcohol, cannabis, and MDMA, also cause persistent disruptions of cognition (Brown et al., 2000; O’Shea, McGregor, and Mallet, 2006; Piper and Meyer, 2004; Stiglick and Kalant, 1982). These findings indicate that the adolescent brain, which is still developing, is susceptible to insult from drug use and abuse, and such insult can result in long-lasting changes in affect and cognition.

DRUGS OF ABUSE AND MENTAL ILLNESS

Drug-related cognitive deficits may be particularly detrimental to the well-being of individuals whose cognitive performance is already compromised by a mental disorder. Moreover, individuals who suffer from mental disorders abuse drugs at higher rates than the general population. Substance abuse is almost twice as prevalent among adults with serious psychological distress or major depressive episodes as among age-matched controls (SAMHSA, 2007, p. 85), and it is estimated that over half of U.S. individuals with drug disorders (excluding alcohol) also have mental disorders (Regier et al., 1990). In a 1986 study, smoking rates approximated 30 percent in population-based controls, 47 percent in patients with anxiety disorder or major depressive disorder, 78 percent in patients with mania, and 88 percent in patients with schizophrenia (Hughes et al., 1986).

The case of smoking and schizophrenia provides one example of a mental disorder that features cognitive deficits in combination with abuse of a drug that causes cognitive decline. As with many comorbidities, effective treatment will likely require untangling the reasons why the two conditions so frequently co-occur:

• Some evidence suggests that patients with schizophrenia smoke to self-medicate. For example, smoking reverses schizophrenic patients’ deficits in the brain’s ability to adapt its responses to stimuli (sensory gating), which could reduce the capacity to filter information, and might account for some of the cognitive disruption seen in the mental disorder. Researchers have traced this feature of schizophrenia to a variant of the gene for the α7 nicotinic acetylcholinergic receptor subunit (Leonard et al., 2001). Consistent with this viewpoint
is an observation that patients smoke less when given the antipsychotic clozapine, which independently alleviates this deficit, than when given haloperidol, which does not (McEvoy, Freudenreich, and Wilson, 1999).

- It has also been proposed that patients with schizophrenia smoke to alleviate side effects of antipsychotic medication (Goff, Henderson, and Amico, 1992). An observation that supports this idea is that patients with schizophrenia smoke more after receiving the antipsychotic haloperidol than when unmedicated (McEvoy et al., 1995).

- Another suggested explanation for the link between smoking and schizophrenia is that smoking itself may precipitate schizophrenia in people predisposed to develop the disease. Among schizophrenics, smokers have an earlier onset of illness, require hospital admissions more frequently, and receive higher doses of antipsychotic medications (Goff, Henderson, and Amico, 1992; Kelly and McCreadie, 1999; Ziedonis et al., 1994).

Another cognitive disorder that is strongly associated with smoking is ADHD. Interestingly, the cognitive symptoms associated with ADHD are similar to those displayed during nicotine withdrawal, and both have been attributed to alterations in the acetylcholinergic system (Beane and Marrocco, 2004; Kenney and Gould, 2008). The high prevalence of smoking among individuals with ADHD (Lambert and Hartsough, 1998; Pomerleau et al., 2003) may be an attempt to self-medicate, because acute nicotine use can reverse some ADHD attentional deficits (Conners et al., 1996). The desire to avoid withdrawal may be a particularly strong motivation for continued smoking in this population, as individuals with ADHD suffer more severe withdrawal symptoms than age-matched controls without the disorder (Pomerleau et al., 2003), and increases in ADHD symptoms following smoking cessation are associated with a greater risk of relapse (Rukstalis et al., 2005). As noted above, however, continued smoking in itself can lead to cognitive decline (Nooyens, van Gelder, and Verschuren, 2008; Richards et al., 2003), and hence might exacerbate ADHD-related symptoms.

Along with nicotine, ADHD is also associated with abuse of stimulants, such as amphetamine and cocaine, and psychoactive drugs, such as cannabis (Elkins, McGue, and Iacono, 2007; Galéra et al., 2008; Tang et al., 2007). Such abuse may also represent attempts at self-medication, as stimulants are used to treat ADHD symptoms (Dopheide and Pliszka, 2009; Kollins, 2008) such as deficits in attention and working memory (Beane and Marrocco, 2004). Some of the distress of ADHD may reflect a reduction in dopaminergic function (Volkow et al., 2009), which might be partially compensated by drugs of abuse (Feltenstein and See, 2008).

**CLINICAL IMPLICATIONS**

The literature reviewed here highlights the importance of considering past and present cognitive function when treating patients for addiction, as drug-related cognitive changes may bias patients toward responses and actions that contribute to the cycle of addiction. Clinicians face the challenge of helping patients master adaptive strategies to overcome the strong associations that contribute to relapse when patients return to environments associated with their prior substance use. In addition, cognitive deficits may hinder patients’ ability to benefit from counseling, and more sessions and/or reminders may be necessary to aid these patients in incorporating
abstinence-sustaining strategies into their daily routines.

Research into the changes in cognition that accompany addiction and the neural substrates of learning and addiction is still in its infancy but has potential to reshape views on addiction. For example, a recent discovery that has generated excitement in the addiction field is that smokers who suffered damage to the insula often lost their desire to smoke (Naqvi et al., 2007). The authors of this finding proposed that the insula is involved in the conscious urge to smoke and that therapies that modulate insula function may facilitate smoking cessation. It may also be that damage to the insula will have a similar effect on the desire to use other drugs of abuse (for a review see Goldstein et al., 2009).

A better understanding of how substances of abuse change cognitive processes is needed to develop new therapeutic agents to treat addiction and ameliorate cognitive deficits. This is a complex issue, however, as different drugs of abuse appear to alter different cognitive processes and cell signaling pathways. Even among users of the same drug, cognitive impacts will differ depending on variations in environmental factors and genetics. Understanding the influence of an individual's genetic background on the manifestation of symptoms is a critical area for future research, holding the promise of informing more effective treatments that can be tailored to the individual's genotype. Finally, understanding how prenatal exposure to drugs of abuse changes neural development should be a high priority, as prenatal exposure increases the new generation's susceptibility to addiction and other problems.

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REFERENCES
Drug addiction encompasses a relapsing cycle of intoxication, bingeing, withdrawal and craving that results in excessive drug use despite adverse consequences (FIG. 1). Drugs that are abused by humans increase dopamine in the reward circuit and this is believed to underlie their rewarding effects. Therefore, most clinical studies in addiction have focused on the midbrain dopamine areas (the ventral tegmental area and substantia nigra) and the basal ganglia structures to which they project (the ventral striatum, where the nucleus accumbens is located, and the dorsal striatum), which are known to be involved in reward, conditioning and habit formation1–3. However, preclinical and clinical studies have more recently brought to light and started to clarify the role of the prefrontal cortex (PFC) in addiction4. A number of processes are ascribed to the PFC that are fundamental for healthy neuropsychological function — encompassing emotion, cognition and behaviour — and that help to explain why PFC disruption in addiction could negatively affect a wide range of behaviours (TABLE 1).

On the basis of imaging findings and emerging preclinical studies5–8, we proposed 10 years ago that disrupted function of the PFC leads to a syndrome of impaired response inhibition and salience attribution (irISA) in addiction (FIG. 1) — a syndrome that is characterized by attributing excessive salience to the drug and drug-related cues, decreased sensitivity to non-drug reinforcers and decreased ability to inhibit maladaptive or disadvantageous behaviours7. As a result of these core deficits, drug seeking and taking become a main motivational drive, occurring at the expense of other activities8 and culminating in extreme behaviours in order to obtain drugs9.

Here we review imaging studies into the role of the PFC in addiction from the past decade, integrating them into the irISA model with the aim to gain a greater understanding of the dysfunction of the PFC in addiction. Specifically, this is the first systematic evaluation of the role of distinct regions within the functionally heterogeneous PFC in the neuropsychological mechanisms that putatively underlie the relapsing cycle of addiction. We review positron emission tomography (PET) and functional MRI (fMRI) studies focusing on regions of the PFC that have been implicated in addiction. These include the orbitofrontal cortex (OFC), anterior cingulate cortex (ACC) and dorsolateral prefrontal cortex (DLPFC) (see TABLE 1 for Brodmann areas; see Supplementary information S1 (table) for Brodmann areas that are not discussed in the main text). We consider the results of these studies (FIG. 2) in the context of the role that the PFC plays in irISA: first, in the response to direct effects of the drug and drug-related cues; second, in the response to non-drug rewards, such as money; third, in higher-order executive function, including inhibitory control; and fourth, in awareness of the illness. We present a simple model that helps to guide our hypotheses regarding the role of the various PFC subregions in the endophenotype of drug addiction (FIG. 3), as described in more detail below. For preclinical studies on the PFC in addiction or in-depth accounts...
into the executive function of the PFC we refer the reader to other reviews. In evaluating this Review, readers need to embrace a myriad of results, which can prove quite confusing as definite conclusions are not always provided. This is particularly true for the localization of functions: for example, are the dorsal ACC and DLPFC involved in the craving response or in control over craving, or in both? Determining which PFC subregion mediates which function can be very difficult, presumably owing to the neuroanatomical and cognitive flexibility of these functions — that is, participants can use multiple strategies when performing neuropsychological tasks, and prefrontal systems seem to have a greater level of functional flexibility than more primary somatomotor systems. Another decade of research may prove invaluable in our understanding of the PFC’s role in drug addiction. Integrating results from preclinical lesion and pharmacological studies, considering other cortical and subcortical structures in addiction — the PFC is densely interconnected with other brain regions (see Box 1 for a discussion of early studies examining these networks in the context of addiction) — and using computational modelling may help further in ascribing probable psychological functions to select PFC regions and in enhancing our understanding of their involvement in drug addiction. Our Review is a step in this direction.

**Direct effects of drug exposure**

Here, we review studies that assessed the effects of stimulant and non-stimulant drugs on PFC activity (Supplementary information S2 (table)). Our model predicts drug-induced enhancements of activity in PFC areas that are involved in drug-related processes — including emotional responses, automatic behaviours and higher-order executive involvement (for example, medial OFC (mOFC) and ventromedial PFC in craving, OFC in drug expectation, ACC in attention bias and DLPFC in forming drug-related working memories). It also predicts drug-induced decreases in non-drug related activity in these same PFC regions, most notably during craving and bingeing in drug-addicted individuals, discussed below (Fig. 3).

Consistent with the former prediction, intravenous cocaine administration to overnight-abstinent cocaine-addicted individuals increased self-reports of high and craving, and mainly increased fMRI blood oxygen level-dependent (BOLD) responses in various PFC subregions. Interestingly, activity in the left lateral OFC, frontopolar cortex and ACC was modulated by drug expectation (that is, activity was greater after expected versus unexpected intravenous delivery of cocaine), whereas subcortical regions responded mainly to the pharmacological effects of cocaine (that is, there was no modulation by expectation); the specific direction of the effect differed by region of interest (ROI). In an 18F-fluorodeoxyglucose PET (PET FDG) study, administration of the stimulant drug methylphenidate (MPH) to active cocaine users increased whole-brain glucose metabolism. Here, the left lateral OFC showed greater metabolism in response to unexpected than to expected MPH; the opposite pattern to that of the BOLD effect in the above study possibly reflects the different temporal sensitivity of the imaging modalities (see below).

Stimulant drugs also increase PFC activity in laboratory animals. For example, regional cerebral blood flow (rCBF) in drug-naive rhesus monkeys increased in DLPFC after non-contingent administration and in ACC during a simple fixed-rate self-administration of cocaine. A PET FDG study in the same animal model showed that cocaine self-administration increased metabolism in OFC and ACC to a greater extent when access to cocaine was extended than when access was limited (note that extended access, but not limited or short access, is associated with transition from moderate to excessive drug intake, as occurs in addiction). Similarly, intracerebroventricular administration of cocaine in rats induced a large fMRI response in selected brain regions, including PFC.

Taken together, the main effect of cocaine (and other stimulants such as MPH) on the PFC is to increase PFC activity, as measured by glucose metabolism, CBF or BOLD (although in a recent study, cocaine reduced PFC cerebral blood volume in macaque monkeys). As the length of access to the drug and drug expectation...
modulate PFC activity, increases in activity that occur during drug administration may be indicative of the neuropsychological processes, including drug-related anticipation (and other conditioned responses), suppress or eclipse non-drug related processes, such as anticipation of—or the motivation to—pursue non-drug related goals (FIG. 3).

In cigarette smokers, rCBF was reduced in the left dorsal ACC (dACC) and this correlated with a decrease in craving after smoking the first cigarette of the day. Similar correlations were reported between rCBF in OFC and craving after acute injections of heroin in people who are heroin-dependent. The disparity between the effects of cocaine (and other stimulants) and other types of drugs on PFC activity may reflect differences in the direct pharmacological effects of the drugs on the PFC and other brain regions (cannabinoid, mu opioid and nicotine receptors, which are targets for marijuana, heroin and nicotine, respectively, have a distinct regional brain distribution) or on non-CNS targets (cocaine and methamphetamine have peripheral sympathomimetic effects that are distinct from the peripheral effects of marijuana or alcohol), or it may reflect variability in methodological factors (for example, whether studies analysed absolute or relative (or normalized) values). It may also be related to drug-induced craving effects: with drugs like cocaine, craving in addicted individuals increases 10–15 minutes after smoking, whereas the studies discussed above reported decreases in craving immediately after nicotine or heroin administration. Viewed in this light, and consistent with our model, the collective results suggest that when drug intake decreases craving, this is associated with decreases in drug-related PFC activity, and vice versa. Concomitantly with these drug-related decreases, we would expect non-drug-related PFC activity to increase, as indeed is the case (see below).

Disparities between results in this section, and throughout this Review, could also be attributed to differences between the various imaging modalities—an issue that should be recognized early on in this Review. For example, PET FDG measures glucose metabolic activity averaged over 30 min, whereas fMRI BOLD and PET CBF reflect faster changes in activation patterns. These modalities also differ in their baseline measures: it is not possible to establish an absolute baseline with BOLD fMRI, whereas it is possible with PET and arterial spin labelling MRI. Another common difference between studies is the baseline state of an individual, for example, the duration of abstinence could impact measures of craving and withdrawal.

### Table 1 | Processes associated with the prefrontal cortex that are disrupted in addiction

<table>
<thead>
<tr>
<th>Process</th>
<th>Possible disruption in addiction</th>
<th>Probable PFC region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Self-control and behavioural monitoring: response inhibition, behavioural coordination, conflict and error prediction, detection and resolution</td>
<td>Impulsivity, compulsivity, risk taking and impaired self-monitoring (habitual, automatic, stimulus-driven and inflexible behavioural patterns)</td>
<td>DLPFC, dACC, IFG and vlPFC</td>
</tr>
<tr>
<td>Emotion regulation: cognitive and affective suppression of emotion</td>
<td>Enhanced stress reactivity and inability to suppress emotional intensity (for example, anxiety and negative affect)</td>
<td>mOFC, vmPFC and subgenual ACC</td>
</tr>
<tr>
<td>Motivation: drive, initiative, persistence and effort towards the pursuit of goals</td>
<td>Enhanced motivation to procure drugs but decreased motivation for other goals, and compromised purposefulness and effort</td>
<td>OFC, ACC, vmPFC and DLPFC</td>
</tr>
<tr>
<td>Awareness and interoception: feeling one’s own bodily and subjective state, insight</td>
<td>Reduced satiety, ‘denial’ of illness or need for treatment, and externally oriented thinking</td>
<td>rACC and dACC, mPFC, OFC and vlPFC</td>
</tr>
<tr>
<td>Attention and flexibility: set formation and maintenance versus set-shifting, and task switching</td>
<td>Attention bias towards drug-related stimuli and away from other stimuli and reinforcers, and inflexibility in goals to procure the drug</td>
<td>DLPFC, ACC, IFG and vlPFC</td>
</tr>
<tr>
<td>Working memory: short-term memory enabling the construction of representations and guidance of action</td>
<td>Formation of memory that is biased towards drug-related stimuli and away from alternatives</td>
<td>DLPFC</td>
</tr>
<tr>
<td>Learning and memory: stimulus–response associative learning, reversal learning, extinction, reward devaluation, latent inhibition (suppression of information) and long-term memory</td>
<td>Drug conditioning and disrupted ability to update the reward value of non-drug reinforcers</td>
<td>DLPFC, OFC and ACC</td>
</tr>
<tr>
<td>Decision making: valuation (coding reinforcers) versus choice, expected outcome, probability estimation, planning and goal formation</td>
<td>Drug-related anticipation, choice of immediate reward over delayed gratification, discounting of future consequences, and inaccurate predictions or action planning</td>
<td>IOFC, mOFC, vmPFC and DLPFC</td>
</tr>
<tr>
<td>Salience attribution: affective value appraisal, incentive salience and subjective utility (alternative outcomes)</td>
<td>Drugs and drug cues have a sensitized value, non-drug reinforcers are devalued and gradients are not perceived, and negative prediction error (actual experience worse than expected)</td>
<td>mOFC and vmPFC</td>
</tr>
</tbody>
</table>

Orbitofrontal cortex (OFC) includes Brodmann area (BA) 10–14 and 47 [REF. 216]; and inferior and subgenual regions of anterior cingulate cortex (ACC) (BA 24, 25 and 32) in the ventromedial prefrontal cortex (vmPFC)⁹; ACC includes rostral ACC (rACC) and dorsal ACC (dACC), respectively, which are included within the medial PFC (mPFC). The mPFC also includes BA 6, 8, 9 and 10 [REF. 218]; dorsolateral PFC (DLPFC) includes BA 6, 8, 9 and 46 [REF. 215]; and the inferior frontal gyrus (IFG) and ventrolateral PFC (vlPFC) encompass inferior portions of BA 8, 44 and 45 [REF. 220]. These various processes and regions participate to a different degree in craving, intoxication, bingeing and withdrawal. IOFC, lateral OFC; mOFC, medial OFC; PFC, prefrontal cortex.
Responses to drug-related cues
At the core of drug addiction are the conditioned responses to stimuli associated with the drug that develop in habitual users — such as objects that are used to administer the drugs, people who procure the drug or emotional states that in the past were either relieved or triggered by the use of the drug — that then drive the desire for drug taking and that are important contributors to relapse. Imaging studies have evaluated these conditioned responses by exposing addicted people to drug-related cues, for example, by showing them drug-related pictures. Here, we first review studies that compared the PFC response to cue exposure in addicted individuals and controls (Supplementary information S3 (table)), and then we discuss studies that explored the effect of abstinence, expectation and cognitive interventions on the PFC responses to drug-related cues (Supplementary information S4 (table)). We predict that in addicted individuals, PFC responses to drug-related cues mimic the responses to the drug itself, owing to conditioning, and that intervention causes a reduction of the drug-cue conditioned responses in the PFC.
Effect of cue exposure on PFC activity. Although there are some exceptions, fMRI studies report that compared to controls, drug-addicted individuals show enhanced BOLD responses in PFC to drug-related cues relative to control cues (Supplementary information S3 (table)).

Figure 3 | A model of PFC involvement in iRISA in addiction. A model of how interactions between prefrontal cortex (PFC) subregions may regulate cognitive, emotional and behavioural changes in addiction. The model shows how changes in the activity of PFC subregions in addicted individuals relate to core clinical symptoms of addiction — intoxication and binging, and withdrawal and craving — compared to PFC activity in healthy, non-addicted individuals or states. The model focuses particularly on inhibitory control and emotion regulation. The blue ovals represent dorsal PFC subregions (including the dorsolateral PFC (DLPFC), the dorsal anterior cingulate cortex (dACC) and the inferior frontal gyrus; see Table 1) that are involved in higher-order control (‘cold’ processes). The red ovals represent ventral PFC subregions (the medial orbitofrontal cortex (mOFC), the ventromedial PFC and rostroventral ACC) that are involved in more automatic, emotion-related processes (‘hot’ processes). Drug-related neuropsychological functions (for example, incentive salience, drug wanting, attention bias and drug seeking) that are regulated by these subregions are represented by darker shades and non-drug related functions (for example, sustained effort) are represented by lighter shades.

a | In the healthy state, non-drug related cognitive functions, emotions and behaviours predominate (shown by the large light-coloured ovals) and automatic responses (emotions and action tendencies that could lead to drug taking) are suppressed by input from the dorsal PFC (shown by the thick arrow). Thus, if a person in the healthy state is exposed to drugs, excessive or inappropriate drug-taking behaviour is prevented or stopped (‘Stop!’). b | During craving and withdrawal, drug-related cognitive functions, emotions and behaviours start to eclipse non-drug related functions, creating a conflict regarding drug taking (‘Stop?’). Decreased attention and/or value is assigned to non-drug related functions (for example, sustained effort) are represented by lighter shades. c | During intoxication and binging, higher-order non-drug related cognitive functions (shown by the small light blue oval) are suppressed by increased input (shown by the thick arrow) from the regions that regulate drug-related, ‘hot’ functions (large dark red oval). That is, there is decreased input from higher-order cognitive control areas (shown by the thin dashed arrow), and the ‘hot’ regions come to dominate the higher-order cognitive input. Thus, attention narrows to focus on drug-related cues over all other reinforcers, impulsivity increases and basic emotions — such as fear, anger or love — are unleashed, depending on the context and individual predispositions. The result is that automatic, stimulus-driven behaviours, such as compulsive drug consumption, aggression and promiscuity, predominate (‘Go!’). This model does not take into account the challenge of localizing PFC functions or the evidence that some addicted individuals use drugs to ‘self-medicate’ in an attempt to normalize PFC functions (although part a could represent an approximation of the normalized PFC functions in these individuals).

These results were reported in the left DLPFC, left medial frontal gyrus and right subcallosal gyrus (Brodman area 34) in young cigarette smokers, and in bilateral DLPFC and ACC in short-term and long-term abstinent alcoholics. Similar increases were reported in studies (including PET FDG studies) of cocaine-addicted individuals watching cocaine-related videos and of heavy smokers watching cigarette-related videos while handling a cigarette. Often, there are no differences between addicted and non-addicted individuals in valence or arousal ratings, or even in autonomic reactions (for example, skin conductance responses) to the drug-related cues, which suggests that neuroimaging measures are more sensitive in detecting group differences in conditioned responses to drug-related cues. Importantly, cue-induced PFC responses were correlated with craving and severity of drug use, and predicted both subsequent performance on a primed emotion recognition task and drug use 3 months later, indicating that these measures have clinical relevance. As no PFC activation was elicited by drug-related masked cues (which activated subcortical regions instead), these effects may only be induced when drug-related cues are consciously perceived, but this needs to be studied further.

An interesting line of studies explores cue-related PFC activation during acute pharmacological drug exposure. In heroin-dependent males receiving heroin injections while viewing drug-related videos, CBF in OFC correlated with the urge to use the drug, and CBF in DLPFC (Brodman area 9) correlated with happiness (Supplementary information S2 (table)). In this context, it is interesting to note that the mere taste of alcohol (versus litchi juice) can increase BOLD PFC activity in young drinkers, and this response correlates with alcohol use and craving and is possibly driven by dopamine neurotransmission in the subcortical reward circuit. By contrast, in non-dependent alcohol drinkers or cigarette smokers, cue-related OFC activity was reduced by alcohol or nicotine administration, respectively. This finding resonates with the finding that in non-addicted subjects, intravenous MPH administration decreased metabolism in ventral PFC regions (BOX 2). Future studies could directly compare PFC responses to drug-related cues in non-dependent and dependent individuals and thereby further explore the impact of intoxication on cue-related PFC responses. Modelling of binging in drug abusing subjects would be informative for the design of interventions to reduce cue-induced compulsive behaviours.
The prefrontal cortex (PFC) is densely interconnected with other cortical and subcortical brain regions and networks, including the ‘default mode network’ (DMN) and the ‘dorsal attention networks’, which are implicated in executive control processes such as attention and inhibition\(^\text{14–15,19–20}\). Although the question of how these networks — and other interconnected brain regions — impact drug addiction has only recently begun to be explored, resting-state functional connectivity studies have already shown promise in revealing patterns that predict disease severity and treatment outcomes. For example, in cigarette smokers, dorsal anterior cingulate cortex (dACC)–striatal connectivity is inversely correlated with the severity of nicotine addiction; using a nicotine patch significantly enhanced the coherence strength of several ACC connectivity paths, including those to frontal midline structures\(^\text{17–18}\). In addition, in abstinent smokers, withdrawal symptom improvement after nicotine replacement therapy was associated with an increased inverse correlation between the executive control network and the DMN, with altered functional connectivity within the DMN, and with altered functional connectivity between the executive control network and regions implicated in reward\(^\text{19,20}\). More recent studies into nicotine addiction adapted an important multi-imaging approach in which connectivity is explored with regard to grey matter integrity and cue reactivity\(^\text{19,20}\).

Network-specific functional connectivity strength is also decreased in other addictions. In cocaine-addicted individuals, the rostroventral ACC (part of the DMN) had lower connectivity with the midbrain, where dopamine neurons are located\(^\text{21–22}\), and similar results have been reported in other studies\(^\text{12–13}\). Reductions in functional connectivity have also been reported in heroin addiction\(^\text{23–24}\), in which connectivity was modulated by drug-related cues\(^\text{25–26}\) and associated with longer duration of heroin use\(^\text{27}\). Further studies are needed to determine whether resting-state connectivity predicts task performance, and how drugs of abuse or potential medications change these measures — for example, does drug administration increase both resting-brain connectivity and task-induced activations or could an elevated resting or baseline state be associated with reduced task-induced activations? These questions are important because the answers will help to determine individually tailored clinical end-points — for example, medication dose could be tapered based on an individual’s baseline resting-state functional connectivity.

Structural imaging studies have shown reduced PFC grey matter density or thickness across addiction populations (up to 20% loss). For example, grey matter PFC decrements, specifically in the dorsolateral PFC (DLPFC), have been documented in individuals who are addicted to alcohol. These decrements are associated with longer lifetime alcohol use\(^\text{28–29}\) and worse executive function\(^\text{30–31}\), and persist from 6–9 months up to 6 years or more of abstinence\(^\text{32–33}\). Despite some conflicting results\(^\text{34–35}\), most studies in individuals who are addicted to cocaine\(^\text{36–37}\), methamphetamine\(^\text{38–39}\), heroin\(^\text{40–41}\) (even when on methadone replacement therapy\(^\text{42–43}\)) and nicotine\(^\text{44–45}\) report similar PFC grey matter reductions — which are most evident in the DLPFC, ACC and orbitofrontal cortex (OFC) — that are associated with longer duration or increased severity of drug use. The persistence of these structural changes beyond the end of drug use and into long-term abstinence suggests an influence of pre-morbid or stable factors that might predispose individuals to drug use and addiction during development (Box 1). Nevertheless, such structural abnormalities are not seen in adolescent users of alcohol\(^\text{46}\) or marijuana\(^\text{47}\), which suggests these PFC decrements could also be a dose-dependent consequence of drug use. Whether it predisposes to addiction or is a consequence of addiction, lower PFC grey matter volume, particularly in the medial OFC, is associated with disadvantageous decision making\(^\text{48\,–\,49}\) that could lead to the catastrophic consequences in the lives of addicted individuals.

Masked cue

A cue that is presented below conscious processing level (that is, outside of conscious awareness). This is usually achieved with a very short duration of cue presentation followed by presentation of another cue that is consciously perceived (longer duration).

Effect of abstinence, expectation and cognitive interventions

Here, we propose that cognitive intervention and long-term abstinence attenuate cue-induced responses in the PFC, and that drug-related expectation and short-term abstinence have the opposite effect. The impact of short-term abstinence on PFC cue-related activity has been most extensively studied in nicotine addiction (Supplementary information S4 (table)). In an arterial spin labelling MRI study, 12-hour abstinence in smokers increased craving, global CBF and regional CBF in the OFC, and decreased CBF in the right PFC, with CBF changes in all ROIs correlating with craving and withdrawal symptoms\(^\text{50}\). Such enhanced cue reactivity was also reported for longer periods of abstinence — up to 8 days in the DLPFC, ACC and inferior frontal gyrus in female smokers\(^\text{51}\) — and also positively correlated with craving\(^\text{52}\). However, some studies report no effect of abstinence on cue-induced PFC activity\(^\text{53}\). This could possibly be attributed to other factors that contribute substantial variability to results, such as the expectation to smoke at the end of the study\(^\text{54}\). Indeed, as discussed above\(^\text{55}\), expectation alone may mimic the effects of acute

left ventromedial PFC BOLD responses to winning versus losing in a gambling-like task, and the size of the reduction was correlated with the severity of the gambling addiction, as assessed with a gambling questionnaire\(^\text{42}\). The opposite directions of the activity changes (hyperactivations versus hypoactivations as compared to controls) may be driven by the ROI (for example, ventromedial PFC task-related deactivations are often seen and have been attributed to the role of the ‘default brain’ network\(^\text{43}\)), differences in craving (craving was reported in REFs 39–41 but not REF 42), task differences or methodological factors, which are summarized at the end of this section.

Disorders that are characterized by impaired control of food consumption are also associated with abnormal PFC reactivity to cues. This is not unexpected, given that these disorders and addiction involve similar compromises in neuronal circuits\(^\text{55}\), including decreased striatal dopamine D2 receptor availability\(^\text{44}\). For example, women with anorexia or bulimia who are passively viewing pictures of foods (versus non-food related pictures) showed increased fMRI BOLD responses in left ventromedial PFC\(^\text{56}\). Compared to patients with bulimia, patients with anorexia showed greater right OFC activation in response to food pictures, possibly implicating this region in overly restrictive self-control; by contrast, left DL-PFC activity to these pictures was decreased in patients with bulimia when compared to healthy controls, possibly implicating this region in the loss of control over food intake\(^\text{57}\). In another study, young women with eating disorders, but not control subjects, showed activation of the left ventromedial PFC during the selection of the most negative word from negative body-image related word sets (compared to during the selection of the most neutral word from neutral word sets)\(^\text{58}\). Such differences were not observed for generally negative words, indicating this region’s activation was driven by words that are most strongly related to the actual concerns of this patient group. Taken together with the results in the pathological gamblers described above\(^\text{59}\), ventromedial PFC responses may track the emotional relevance of cues of highest concern to the patient population in question (that is, winning or avoiding loss for individuals with pathological gambling, body image for individuals with eating disorders and drug-related cues for drug-addicted individuals) and could serve as a target for tracking therapeutic interventions in addiction, as was recently suggested\(^\text{40,49}\).
drug intake on PFC activation in addicted individuals. Studies in which all three variables — expectation for drug administration, exposure to drug-related cues and abstinence — are explored for main effects and interaction effects on PFC activity would be useful, particularly if they involve large samples. The temporal dynamics of PFC cue reactivity also remain to be explored in longitudinal studies, tracking the same individual throughout longer-term abstinence periods.

A promising line of research explores behavioural modulation of cue reactivity. For example, a role for the mOFC in the suppression of craving was suggested by findings from a recent PET study in cocaine users. Craving increased after watching a video of cocaine-related cues, and craving levels correlated with glucose metabolism in the medial PFC. Importantly, when participants were instructed — before watching the video — to inhibit craving, metabolism in the right mOFC decreased, and this was associated with activation of the right inferior frontal gyrus (Brodmann area 44), which is a crucial region in inhibitory control. In treatment-seeking cigarette smokers, the instruction to resist craving while viewing smoking-related videos was associated with DLPFC and ACC activation, although unexpectedly, this activation correlated positively with craving.

A recent study suggests that the direction of the change in activity and correlation with craving may be modulated by the behavioural strategy that is used to suppress craving. In this elegant study, cigarette smokers were instructed to consider the immediate versus long-term consequences of consuming the stimuli depicted in pictures (cigarette-related versus food-related cues). Considering the long-term consequences was associated with increased activity in PFC regions associated with cognitive control (DLPFC and inferior frontal gyrus) and with decreased activity in PFC regions associated with craving (mOFC and ACC). In addition, self-reported craving decreased when subjects considered the

### Box 2 | The role of dopamine and other neurotransmitters

**Dopamine D2 receptors**, which are most densely expressed in subcortical regions such as the midbrain and dorsal and ventral striatum, are also distributed throughout the prefrontal cortex (PFC). A series of positron emission tomography (PET) studies reported lower striatal dopamine D2 receptor availability in individuals who are addicted to methamphetamine, cocaine or alcohol, and in people with morbid obesity, and these reductions were associated with decreased baseline metabolic activity in the orbitofrontal cortex (OFC) and anterior cingulate cortex (ACC). This suggests that loss of dopamine signalling through D2 receptors may underlie some of the deficits in prefrontal function that are seen in addiction — an idea that is supported by preliminary data showing that striatal dopamine D2 receptor availability was correlated with medial PFC response to money in cocaine-addicted individuals. Reduced striatal dopamine D2 receptor availability was also reported in male heavy smokers, both after smoking as usual and after 24 hours of abstinence; in the sated condition, the dopamine D2 receptor availability in the bilateral ACC was negatively correlated with the desire to smoke (positive correlations were observed for the striatum and OFC). Evidence for dopamine depletion in the dorsolateral PFC (DLPFC) was also reported in young chronic ketamine users, and levels of depletion were correlated with higher weekly drug use. Other PET studies reported markedly attenuated striatal dopamine release in response to intravenous administration of a stimulant drug (for example, methylphenidate) in cocaine abusers and alcoholics, with a parallel decrease in self-reported experiences of feeling high.

Consistent with data from animal studies, these results in addicted individuals point to a blunted striatal dopaminergic function — both at baseline and in response to a direct challenge — that is associated with enhanced craving and severity of use. A blunted striatal dopamine response is predictive of actual choice for cocaine over money in abstinent cocaine-addicted individuals, suggesting that it may predispose subjects to relapse. The results also suggest that, by regulating the magnitude of dopamine increases in the striatum, the OFC assumes a crucial role in the modulation of the value of reinforcers; disruption of this regulation may underlie the increased value attributed to a drug reward in addicted subjects. Consistent with this suggestion, metabolism in the medial OFC and ventral ACC in cocaine abusers increased after intravenous stimulant administration, whereas it was reduced in controls; the regional metabolic increases in the abusers were associated with drug craving.

Endogenous opioids also mediate the rewarding responses of many drugs of abuse, particularly heroin, alcohol and nicotine. Repeated drug use has been associated with decreased release of endogenous opioids, an effect that may contribute to withdrawal symptoms, including dysphoria. A study using radioligands showed that cocaine abusers had higher PFC µ opiate receptor binding potential (indicative of lower endogenous opioid levels) than healthy non-addicted controls, and this persisted in the anterior frontal cortex and ACC throughout 12 weeks of abstinence. Elevated µ opiate receptor binding in the DLPFC and ACC before treatment was associated with greater cocaine use and shorter duration of abstinence, and was suggested to be a better predictor of treatment outcome than baseline drug and alcohol use. Similar results were reported in abstinent alcoholic men, whereas the level of µ (or kappa) opiate receptor binding is reversed by chronic methadone in heroin-addicted individuals.

Decreased PFC binding potential for a serotonin transporter radioligand has been reported in abstinent methamphetamine abusers, young recreational MDMA users and in recovered alcoholics. Reduced serotonin transporter availability may reflect neuroadaptations to increased synaptic serotonin, but it could also reflect damage to serotonergic nerve terminals. Other neurotransmitter systems that regulate the PFC and are involved in the neuroadaptations that occur with repeated drug use in laboratory animals include the glutamate and the cannabinoid systems. However, so far there are no published studies with radiotracers to image these systems in human addiction.

See Supplementary information (table) for an overview of studies comparing neurotransmitter systems between addicted individuals and healthy controls.
Studies on how pre-morbid vulnerabilities — such as prenatal exposure to drugs, family history or selected gene polymorphisms and their interactions — impact prefrontal cortex (PFC) function are crucial for the design of future intervention and possibly prevention efforts; these studies highlight the importance of targeting clear biomarkers of vulnerability to drug use and addiction. For example, reduced absolute global cerebral blood flow (CBF) (<10%), and enhanced relative CBF in the dorsolateral PFC (DLPFC) (9%) and anterior cingulate cortex (ACC) (12%) were reported in adolescents with heavy prenatal cocaine exposure291. A hyperactive PFC was also reported in young users of MDMA202, marijuana203 or alcohol204 during the go/no-go task, in which they performed normally (Supplementary information S6 (table)). Similarly, compared to control children and children who had alcoholic parents but were resilient, children who had alcoholic parents and were vulnerable to alcohol drinking (classified based on the level of problem drinking over the course of adolescence) had a hyperactive right dorsolateral PFC, while the bilateral orbitofrontal cortex (OFC) was hypoactive, despite a lack of behavioural differences when silently reading emotional words. Across the entire sample, such dorsolateral PFC hyperactivity was associated with more externalizing symptoms and with aggression205 (Supplementary information S5 (table)). Thus, such changes in PFC activity may be compensatory in the short-term (as evidenced by equal task performance), but in the long-term may promote substance abuse and addiction in these individuals, although this remains to be ascertained.

The mechanism that underlies such vulnerability to, or that confers protection against, developing addiction may involve altered dopaminergic neurotransmission. For example, striatal dopamine D2 receptor availability and regional PFC metabolism were higher in young, unaffected members of alcoholic families than in subjects without such family history, which is the opposite to results commonly reported in addicted individuals (BOX 2; see Supplementary information S2 (table)). The individuals with a family history of alcohol abuse reported lower positive emotionality, and this was associated with both lower striatal dopamine D2 receptor availability and lower OFC metabolism. It is therefore possible that the higher dopamine D2 receptor availability and the enhanced metabolic activity in PFC in individuals with a family history of alcohol abuse increased the level of positive emotionality — although this nonetheless remained below the level in healthy controls — to levels that may have protected these individuals against developing addiction. It is also possible that optimal conditions are needed for the maintenance of such protection, and that suboptimal conditions (for example, chronic stress) could expose these same individuals to addiction later in life, but this remains to be determined in longitudinal studies. Other mechanisms, such as brain dysmorphology206, may also be important in conferring vulnerability to addiction.

Genetic contributions to vulnerability to addiction are also important. For example, regular marijuana users with risk alleles of genes that encode the cannabinoid receptor 1 (CB1) or the fatty acid amino hydrolase 1 (FAAH; the enzyme that metabolizes endogenous cannabinoids) had greater drug-related cue reactivity in receptor 1 (CB1) or the fatty acid amino hydrolase 1 (FAAH; the enzyme that metabolizes endogenous cannabinoids)207, may also be important in conferring vulnerability to addiction.

Box 3 | Vulnerability and predisposition to drug use

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In this interpretation, frequent and high-dose drug use leads to compensatory brain changes that limit appetitive hedonic and motivational processes (‘reward’), instead strengthening aversive (opponent or ‘anti-reward’) systems. This process is similar to tolerance, in which sensitivity to reward is decreased. It is also captured by the opponent-process hypothesis set forth by Sloman and Corbit, which describes the temporal dynamics of opposing emotional responses; here, negative reinforcement (for example, withdrawal) prevails over positive reinforcement (for example, drug-induced high) in the transition from occasional drug use to addiction. This process is relevant to emotional reactivity and emotion regulation, which, insofar as emotions are defined as ‘states elicited by reinforcers’, are bound to be impaired in drug addiction, especially during drug-biased processing such as craving and bingeing.

Anhedonia is a defining characteristic of drug dependence, and criteria for major depressive disorder — which includes anhedonia as a core symptom — are met by many drug-addicted individuals (for example, 50% of cocaine-addicted individuals). The strong association between mood and substance use disorders is not limited to depression; for example, emotional distress is a risk factor for drug relapse. However, research on how altered emotion processing is implicated in substance use disorders is in its infancy, as discussed below (Supplementary information S2  table).

Money is an effective abstract, secondary and generalizable reinforcer that acquires its value by social interaction, and it is used in emotional learning in everyday human experience; compromised processing of this reward may therefore point to a socially disadvantageous emotional learning mechanism in addiction. Such a deficit, all the more distinct given the strong motivational and arousal value that is normally associated with this reward, would corroborate the idea that in addiction, brain reward circuits are ‘hijacked’ by drugs, although the possibility for a pre-existing deficit in reward processing also cannot be ruled out.

One fMRI study investigated how cocaine-addicted individuals and controls responded to receiving monetary reward for correct performance on a sustained attention and forced-choice task. In controls, sustained monetary reward (gain that did not vary within task blocks and that was fully predictable) was associated with a trend for the left lateral OFC to respond in a graded fashion (activity monotonically increased with amount: high gain > low gain > no gain), whereas the DLPFC and rostral ACC responded equally to any monetary amount (high or low gain > no gain). This pattern is consistent with the OFC’s role in processing relative reward, as documented in non-human and human subjects, and with the DLPFC’s role in attention. Cocaine-addicted subjects showed reduced fMRI signals in left OFC for high gain compared to controls and were less sensitive to differences between monetary rewards in left OFC and in DLPFC. Remarkably, more than half of the cocaine-addicted subjects rated the value of all monetary amounts equally (that is, US$10 = US$1000). Eighty-five percent of the variance in these ratings could be attributed to the lateral OFC and medial frontal gyrus (and amygdala) responses to monetary reward in the addicted subjects. Although these findings need to be replicated in a larger sample size and with more sensitive tasks, they nonetheless suggest that some cocaine-addicted individuals may have reduced sensitivity to relative differences in the value of rewards. Such ‘flattening’ of the perceived reinforcer gradient may underlie over-valuation or bias towards immediate rewards (such as an available drug) and the discounting of greater but delayed rewards, therefore reducing sustained motivational drive. These results may be therapeutically relevant as monetary reinforcement in well-supervised environments has been shown to enhance drug abstinence, and may also be relevant in predicting clinical outcomes. In line with this idea, in a similar population of subjects, the degree of DACC hypoactivation in a task in which correct performance was monetarily remunerated correlated with frequency of cocaine use, whereas degree of rostroventral ACC (extending to mOFC) hypoactivation correlated with task-induced craving suppression. There was an inverse association of these PFC ROIs with cue reactivity in the midbrain in cocaine-addicted subjects but not in control subjects, which implicates these ACC subdivisions in the regulation of automatic drug responses.

It should be noted that in the studies described above, subjects were not asked to choose between monetary rewards. We predict that choice would similarly follow a linear function (choice of higher over lower reward) in healthy controls more so than in addicted individuals, who we expect to show less flexibility in choice (choosing drug over other reinforcers), particularly during craving and bingeing. Studies that allow subjects to choose between reinforcers have mostly been conducted in laboratory animals. These studies have shown that, when given the choice, previously drug-exposed animals choose the drug over novelty, adequate maternal behaviour and even food, indicating that drug exposure can decrease the perceived value of natural rewards, even those that are needed for survival. In a recent human neuroimaging study in which subjects could win cigarettes or money, occasional smokers were more motivated to obtain money than cigarettes, whereas dependent smokers made similar efforts to win money or cigarettes. A similar group by reward interaction was observed in the right OFC, bilateral DLPFC and left ACC, such that in the occasional smokers these regions showed higher activity to stimuli predicting an increasing monetary reward than to stimuli predicting a cigarette reward, whereas the dependent smokers showed no significant differences in such anticipatory brain activity. These regions also showed higher activation to money in the occasional than in dependent smokers.

These results, together with behavioural results on neuropsychological tests in cocaine-addicted individuals (see also BOX 2), contribute to our understanding of how relative reward preferences may change in addiction such that preference for the drug competes with (and sometimes exceeds) preference for other...
reinforcers, with a concomitant decrease in the ability to assign relative values to non-drug-related rewards.

**Emotional reactivity.** Several studies that are reviewed above compared PFC responses to non-concern-specific yet emotionally arousing stimuli with responses to concern-related (for example, drug-related) cues.\(^{25,26,28,46,47}\) (Supplementary information S3 (table)). The PFC was hyperactive in response to images from all emotional categories in alcohol-addicted subjects,\(^{28}\) the anterior PFC was hypoactive in response to pleasant pictures in heroin-addicted individuals,\(^{26}\) and in patients with eating disorders PFC responses to aversive pictures were normal.\(^{16,47}\) Thus, in contrast to our model’s predictions (FIG. 5), there were no differences in the PFC response between drug-related and affective yet non-drug-related cues in any of these studies. This result, and the variability in the pattern of results, could be attributed to — among other factors — the small number of studies, differences between studies (such as sample sizes, the primary drug of abuse and duration of abstinence) and sensitivity of the measures used. Future studies would benefit from using event-related potential recordings or electroencephalography, which have much higher temporal resolution than fMRI or PET.

A clearer picture emerges when studies incorporate emotional processing into cognitive–behavioural tasks (Supplementary information S5 (table)). For example, when required to empathize with a protagonist in a series of cartoons, each depicting a short story, methamphetamine-addicted individuals provided fewer correct answers than controls to the question “what will make the main character feel better?”\(^{29}\) Compared to control subjects, the addicted individuals also showed hypoactivation in OFC (and hyperactivation in DLPFC) when answering this question. With the exception of one study in abstinent heroin-addicted individuals,\(^{44}\) other similar studies also reported differences between addicted and control groups in PFC responses to tasks requiring processing of emotional stimuli such as faces, words or complex scenes. For example, when men with alcohol addiction judged the intensity of five facial expressions, negative expressions were associated with lower activations in the left ACC but higher activations in the left DLPFC and right dACC compared to controls.\(^{30}\) In addition, compared to healthy controls, cocaine users showed ACC and dorsomedial PFC hypoactivations while performing a letter discrimination task during the presentation of a set of pleasant (versus neutral) pictures and hyperactivations in the bilateral DLPFC during the presentation of unpleasant (versus pleasant) pictures.\(^{30}\) Similarly, compared to healthy controls, marijuana smokers showed left ACC hypoactivations, and right DLPFC and inferior frontal gyrus hyperactivations in response to presentation of masked angry faces (versus neutral faces); right ACC responses positively correlated with frequency of drug use and bilateral ACC responses correlated with urinary cannabinoid levels and alcohol use.\(^{30}\) By contrast, the left dACC was hyperactive in methamphetamine-dependent subjects compared to controls when judging emotional expression on faces in an affect matching task (versus judging the shape of abstract figures) and this was associated with more self-reported hostility and interpersonal sensitivity in the addicted subjects.\(^{30}\)

Taken together, these studies indicate that the DLPFC is mostly hyperactive during emotion processing in addicted individuals compared to control subjects, especially for negative emotions. The ACC shows mixed results, although with more studies showing hypoactivity than hyperactivity. It is possible that the DLPFC hyperactivity may be compensating for the ACC hypoactivity, which would explain the lack of difference in task performance between drug abusers and healthy controls in most of these studies. Disadvantageous and/or impulsive behaviours may be observed during greater emotional arousal challenges such as stress, craving or more difficult tasks. Clearly, the roles of these regions in relation to the proposed model (FIG. 3) need to be better understood. It is possible that, by prematurely recruiting higher-order PFC executive function (mediated by the DLPFC), negative emotional arousal enhances risk for drug use in addicted individuals, particularly in situations that place additional strain on the limited cognitive control resources. This interpretation is consistent with the competition between drug and non-drug-related processes and between ‘cold’ and ‘hot’ processes in the model (FIG. 5c).

Although several of the above studies used negatively valenced stimuli, a lingering question is whether altered sensitivity to non-drug reinforcers in addicted individuals also applies to negative reinforcers such as money loss. Studies in animals show that ‘addicted’ subjects manifest persistent drug seeking even if the drug is associated with receiving an electric shock.\(^{30}\) In humans, hypoactivation in the right ventrolateral PFC in smokers during monetary loss, and in gamblers during monetary gain, have been reported\(^ {100}\) (Supplementary information S5 (table)). Although more studies are clearly needed, the implication of reduced sensitivity to negative reinforcers in addiction has practical implications as, in addition to positive reinforcers (such as vouchers and privileges), negative reinforcers (such as incarceration) are increasingly being used in the management of drug abusers. Interventions could be optimized by selecting the most effective type and dose of reinforcer. Future studies could also help to ascertain whether addicted individuals may resort to taking drugs because they are easily bored, frustrated, angry or fearful, perhaps as a result of altered PFC functioning. Low threshold for experiencing any of these emotions, or the inability to sustain goal-directed behaviour (for example, completing a boring task) when experiencing these emotions, may be associated with impaired inhibitory control (that is, enhanced impulsivity) as reviewed below. In cocaine-addicted individuals, PFC activity habituates prematurely to repeated presentation of an incentive sustained attention task\(^ {101}\), which could be a measure of compromised sustainability of effort and result in inadequate engagement in treatment activities.
Inhibitory control in addiction

Drug addiction is marked by mild, yet pervasive, cognitive disruptions92 that may accelerate its course, threaten sustained abstinence103 or increase attrition from treatment104,105. The PFC is essential for many of these cognitive processes, including attention, working memory, decision making and delay discounting (Table 1), all of which are compromised in addicted individuals, as reviewed elsewhere106. Another important cognitive function of the PFC is self-control, and here we focus on the role of the PFC in this process in addiction (Supplementary information S6 (table)). Self-control refers, among other operationalizations, to a person’s ability to guide or stop a behaviour, particularly when the behaviour may not be optimal or advantageous, or is perceived as the incorrect thing to do. This is pertinent to addiction as, despite some awareness of the devastating consequences of drugs (see also the section below on disease awareness in addiction), individuals who are addicted to drugs show an impaired ability to inhibit excessive drug taking. Impaired inhibitory control, which is a key operation in self-control, is also likely to contribute to engagement in criminal activities in order to procure the drug, and to underlie the impaired regulation of negative emotions, as suggested above. These impairments could also predispose individuals to addiction. Consistent with previous reports107, children’s self-control during their first decade of life predicts substance dependence in their third decade of life108.

Go/no-go task

A neuropsychological task that is commonly used to assess inhibitory control. Subjects are required to press a button when one stimulus type appears and withhold a response when another stimulus type appears.

Stop signal reaction time task (SSRT). A neuropsychological test that measures the ability to stop a response that has already been initiated. It is used clinically as an index of inhibitory control. Slower SSRT is associated with disruption of executive functions.

Errors of omission and commission

Errors on a go/no-go task. A subject had to go but they did not go (omission of a response) or had to withhold a response but pressed a button instead (commission of an unnecessary response). The former is an index of inattentiveness while the latter is an index of impulsive (premature) responding.

Stroop task

A neuropsychological task in which conflict is created between an automatic response (for example, reading) and a slower response (for example, colour naming), with both competing for the same processing resources. Impaired performance on Stroop tasks is associated with prefrontal cortex dysfunction.

Go/no-go and stop signal reaction time tasks. Tasks that are often used to measure inhibitory control are the go/no-go task and the stop signal reaction time task (SSRT). In the go/no-go task, cocaine-addicted individuals showed more errors of omission and commission than controls and this has been attributed to hypoaivation in dACC during stop trials109. In another study, this inhibitory behavioural deficit in cocaine users was exacerbated by a higher working-memory load; again, dACC hypoaivation was associated with deficient task performance110. Similarly, heroin-addicted men showed slower reaction times in the go/no-go task, along with hypoaivation in ACC and medial PFC111. Results from the SSRT are more difficult to interpret. For example, the ACC was hypoaive during successful response inhibitions compared to failed response inhibitions in cocaine-addicted men, and their behavioural performance was similar to that of controls112. The ACC was also hypoaive during both careful behavioural adjustment and risk taking on this task in abstinent alcoholics, particularly in subjects with higher alcohol urge at the time of the fMRI scan113. By contrast, the ACC was hyperactive during inhibition errors114, possibly because the abstinent alcoholics exercised a greater attention in monitoring for the stop signal than controls — a function that is associated with the ACC. Increased activity in other regions of the PFC was also reported in cigarette smokers after a 24-hour abstinence, but (in contrast to expectation for an increased regional activation) accuracy was reduced114 (Supplementary information S4 (table)).

The large variability in results from these studies is possibly caused by differences in the analyses, the type of comparison and by performance differences between the groups, in addition to other variables. Nevertheless, a pattern emerges in which the dACC is hypoaive during these inhibitory control tasks, and this hypoaivity is mostly associated with impaired performance, particularly with shorter abstinence durations. Targeted cognitive–behavioural interventions may alleviate this dysfunction. For example, informative cueing (such as providing a warning of an impending no-go trial) enhanced inhibitory control in a go/no-go task, and this was correlated with enhanced ACC activation in methamphetamine-addicted individuals115. Such cognitive–behavioural interventions could be used as neural rehabilitation exercises and combined with the simultaneous administration of drugs, as discussed below.

Stroop tasks. Inhibitory control can also be assessed using the colour–word Stroop task116. Slower performance and more errors during incongruent trials on this task are a hallmark of PFC dysfunction. Neuroimaging research has shown that the dACC and DLPFC are involved in this task117,118, with distinct roles for these regions in conflict detection (dACC) and resolution (DLPFC)119.

Studies using the colour–word Stroop task in addicted individuals report results that mostly echo those reported above. For example, cocaine abusers had lower CBF in the left dACC and right DLPFC during incongruent trials compared to congruent trials, whereas the right ACC showed the opposite pattern; moreover, right ACC activation was negatively correlated with cocaine use120 (Supplementary information S6 (table)). In marijuana–using men, lower CBF during this task was reported in several PFC regions, including perigenual ACC, ventromedial PFC and DLPFC121. Methamphetamine-dependent subjects also showed hypoaivistions in the inhibitory control network, including dACC and DLPFC while performing this task122. Consistent with the impact of abstinence on the go/no-go task reported above123, cigarette smokers who were tested after a 12-hour abstinence had slowed reaction times, and enhanced dACC and reduced right DLPFC responses to the incongruent trials on the colour–word Stroop task124 (Supplementary information S4 (table)). Importantly, an fMRI study showed that activation of the ventromedial PFC (Brodmann areas 10 and 32) during a colour–word Stroop task performed 8 weeks before treatment onset predicted treatment outcome in cocaine-addicted individuals125.

In the emotional variant of this task, colour words are substituted for emotional words or pictures that are related to a particular individual’s area of concern, such as alcohol-related words for alcohol-addicted individuals. Although both the classic and the emotional Stroop tests involve the need to suppress responses to distracting stimulus information while selectively maintaining attention on the stimulus property that is needed to complete the task, only the emotional Stroop task uses emotional relevance as a distractor. Such emotional
Stroop designs can potentially further demarcate the altered PFC activity in addiction: is it generalizable to any type of conflict or does it occur specifically during conflicts in a drug-related context?

An fMRI study in stimulant users showed attention bias to drug-related words: addicted individuals, but not controls, showed more attention bias to drug-related words (measured as the median response latency of correctly identified colours of drug-related words minus the median response latency of correctly identified colours of matched neutral words), which was correlated with enhanced left ventral PFC responses. Such responses were not observed for the colour–word Stroop task. Similarly, drug-related pictures amplified dACC responses to task-relevant information in cigarette smokers. These findings suggest that in addiction, more top–down resources are needed to focus on cognitive tasks when drug-related cues are present as distractors (thus biasing attention) during the task. Conflicting with these and other results are studies in current cocaine users, in which drug-related words were not associated with slower performance or more errors. This disparity could be related to task design or the treatment-seeking status of the study participants; we predict that enhanced conflict between drug-related words and neutral words characterizes those individuals who are trying to abstain from drugs. Evidence for such an effect in cigarette smokers was recently published.

Effects of drug administration during inhibitory control tasks. Deficits in emotion regulation and inhibitory control in addicted individuals and enhancement of PFC activity by direct drug administration (see above and Supplementary information S2 (table)) together could support the self-medication hypothesis. According to this hypothesis, drug self-administration — and the associated increases in PFC activity — ameliorate the emotional and cognitive deficits that are present in drug-addicted individuals. Such a self-medication effect has previously been recognized by the treatment community, as evidenced by using methadone (a synthetic opioid) as a standard agonist substitution therapy for heroin addiction. An fMRI study in stimulant users showed attention bias to drug-related words: addicted individuals, but not controls, showed more attention bias to drug-related words (measured as the median response latency of correctly identified colours of drug-related words minus the median response latency of correctly identified colours of matched neutral words). This was correlated with enhanced left ventral PFC responses. Such responses were not observed for the colour–word Stroop task. Similarly, drug-related pictures amplified dACC responses to task-relevant information in cigarette smokers. These findings suggest that in addiction, more top–down resources are needed to focus on cognitive tasks when drug-related cues are present as distractors (thus biasing attention) during the task. Conflicting with these and other results are studies in current cocaine users, in which drug-related words were not associated with slower performance or more errors. This disparity could be related to task design or the treatment-seeking status of the study participants; we predict that enhanced conflict between drug-related words and neutral words characterizes those individuals who are trying to abstain from drugs. Evidence for such an effect in cigarette smokers was recently published.

A PET study showed that oral MPH attenuated the reduced metabolism in limbic brain regions — including lateral OFC and DLPFC — that followed exposure to cocaine-related cues in cocaine-addicted individuals. It also decreased errors of commission, a common measure of impulsivity, during a drug-relevant emotional Stroop task, both in cocaine-addicted individuals and controls, and in the addicted individuals this decrease...
was associated with normalization of activation in the rostroventral ACC (extending to the mOFC) and dACC; dACC task-related activation before MPH administration was correlated with shorter lifetime alcohol use137 (FIG. 4). Although it remains to be studied whether or how the noradrenergic effects of MPH contribute to its ‘normalizing’ effects in cocaine users, taken together these results suggest that the dopamine-enhancing effects of MPH could be used to facilitate changes in behaviour in addicted individuals (for example, improve self-control), particularly if MPH treatment is combined with specific cognitive interventions.

It should be noted that the effect of dopamine agonists on normalizing brain–behaviour responses to emotional or cognitive–control challenges may depend on patterns of compulsive drug use138 or other individual differences, such as baseline self-control and lifetime drug use, but these possibilities remain to be studied in larger sample sizes. Also, non-dopaminergic probes (for example, cholinergic or AMPA receptor agonists) may offer additional pharmacological targets for cocaine addiction treatment139.

In summary, results of studies into inhibitory control in drug addiction suggest that there is dACC hypoactivity and deficient inhibitory control in drug-addicted individuals. Enhanced PFC activity has been reported after short-term abstinence, upon exposure to drug-related cues and to the drug itself (or similar pharmacological agents). However, although drug exposure is also associated with better performance in these cognitive tasks, short-term abstinence and exposure to drug-related cues have the opposite result on task performance. Viewed in the context of the proposed model (FIG. 3), although drugs of abuse offer temporary relief, chronic self-medication with these drugs has long-term consequences — reduced inhibitory control mechanisms and associated emotional disruptions — that may not be alleviated with short-term abstinence, and that are prone to be rekindled upon exposure to drug-related cues. Normalizing these functions, using empirically based and targeted pharmacological and cognitive–behavioural interventions — in combination with the relevant reinforcers — should become a goal in the treatment of addiction.

Disease awareness in addiction

The capacity for insight into our internal world (encompassing interoception but extending to higher-order emotional, motivational and cognitive self-awareness) is partly dependent on the PFC. Given the impairments in PFC function in people with addiction reviewed above, it is possible that a restricted awareness of the extent of the behavioural impairment or of the need for treatment may underlie what has traditionally been ascribed to ‘denial’ in drug addiction — that is, the assumption that the addicted patient is able to fully grasp his or her deficits but chooses to ignore them may be erroneous. Indeed, studies have recently suggested that addicted individuals are not fully aware of the severity of their illness (that is, their drug seeking and taking behaviour and its consequences) and this may be associated with deficits in the control network139.

Several studies have provided evidence for a dissociation between self-perception and actual behaviour in addiction. For example, in healthy controls the speed and accuracy of responses for a high monetary condition compared to a neutral cue in a monetarily remunerated forced-choice sustained attention task was correlated with self-reported engagement in the task; by contrast, cocaine subjects’ reports of task engagement were disconnected from their actual task performance, indicating discordance between self-reported motivation and goal-driven behaviour93. Using a recently developed task in which participants selected their preferred pictures from four types of pictures and then reported what they thought was their most selected picture type94, the discordance between self-report and actual choice — indicating impaired insight into one’s own choice behaviour — was most severe in current cocaine users, although it was also discernible in abstinence users, in whom it was correlated with frequency of recent cocaine use95.

An underlying mechanism of this dissociation may be an uncoupling of behavioural and autonomic responses during reversal learning, such as has been shown to occur after OFC lesioning in monkeys96. There is some evidence for similar neural–behavioural dissociations also in humans. In an event-related potential study using the task reported above97, control subjects showed altered electrocortical responses and reaction times in the high-money condition compared to the neutral cue condition, and these two measures of motivated attention were intercorrelated. This pattern was not observed in the cocaine-addicted group, in which the ability to respond accurately to money (that is, the more the behavioural flexibility to this reinforcer), negatively correlated with the frequency of recent cocaine use98.

Another study showed that, in a gambling task, control subjects’ choices were guided by both actual and fictive errors, whereas cigarette smokers were only guided by the actual errors that they had made, even though the fictive errors induced robust neural responses99, again pointing to neural–behavioural dissociations in addiction. In the proposed model (FIG. 3), this mechanism is represented by a decreased input from higher-order cognitive control regions to regions that are associated with emotional processing and conditioned responses.

Importantly, in humans this neural–behavioural dissociation can be validated by comparing patients’ self-reports with those of informants100 such as family members or treatment providers, or with objective measures of performance on neuropsychological tests101. It is important to remember that self-report measures provide an important glimpse into such dissociations, but given the limitations of self-reports, the development of more objective measures of insight and awareness is crucial for both research and clinical purposes. Two promising measures are error awareness and affect matching. Error awareness in a go/no-go task was found to be reduced in young marijuana abusers and this was associated with reductions in bilateral DLPFC and right ACC, and with greater current drug use102. In methamphetamine-dependent subjects, the bilateral ventrolateral PFC was hypoactive during affect matching and this...
was associated with more self-reported alexithymia. As better awareness of the severity of drug use predicted actual abstinence for up to 1 year after treatment in alcoholics, this budding line of research could greatly enhance our understanding of relapse in drug addiction, potentially improving currently available intervention approaches, for example, by targeting addicted individuals who have reduced self awareness for tailored interventions.

Study limitations and future directions
The main limitation of this Review is our selective focus on the PFC at the expense of excluding all other cortical brain regions and subcortical structures. The architecture supporting higher-order executive function and top-down control is complex and is thought to involve several functional networks that include, in addition to the PFC, other regions such as the superior parietal cortex, insula, thalamus and cerebellum. Consequently, and also given the inherent limitations of cross-sectional human neuroimaging studies, attribution of causality should be avoided — that is, the PFC may not directly drive the deficits described in this review. Future meta-analyses in which the disruption of these functional networks in addiction is explored should be imbued with results from mechanistic studies in laboratory animals.

A notable issue with many of the reviewed studies pertains to their use of functional ROI analyses that sometimes lack the more stringent statistical corrections of whole-brain analyses. For example, to overcome issues of low power, reported results are sometimes restricted to post-hoc analyses in regions that showed significant results across all subjects to all task conditions; whole-brain analyses of the main (for example, group or type of stimulus) or interaction effects, or of correlations with task performance or clinical end-points, are not consistently performed. Therefore, such ROI results could represent a Type I error but they could also miss the key neural substrates that are involved in the phenomenon under investigation, for example, craving or control of craving. A way to circumvent the limitations of post-hoc analyses is to perform both whole-brain analyses and use a priori defined anatomical ROIs, which could also help to standardize the nomenclature of ROIs across studies. Other common issues pertain to incomplete presentation of the actual data (such as not providing both mean and variance, or not providing scatterplots when reporting correlations), which can obscure the direction of an effect (activation versus deactivation), potentially adding to the variability in published results (for example, a hyperactivation could refer to higher activations or lower deactivations from baseline). In summary, this field would benefit from standardization — of procedures related to imaging, tasks, analyses and subject characterization — that would facilitate the interpretability of the findings. Standardization is also crucial for allowing integration of data sets from various laboratories — such data pooling will be particularly important for genetic studies that are aimed at understanding the interplay between genes, brain development, brain function and the effects of drugs on these processes. For example, the creation of large imaging data sets are going to be important in understanding how genes that are associated with vulnerability for addiction affect the human brain both after acute and repeated drug exposures. Moreover, the ability to integrate large imaging data sets — as has recently been done for MRI images of resting functional connectivity — will allow a better understanding of the neurobiology of addiction that in the future may serve as biomarker to guide treatment.

Although there are a few exceptions (implicating the right PFC, particularly the ACC and DL-PFC, in compensatory inhibitory processes) the data reviewed here show no clear pattern indicating lateralization of brain changes in addicted individuals. However, lateralization was not the focus of investigation in any of the reviewed studies. Given that there is evidence for disrupted laterality during finger-tapping in cocaine abusers, studies that specifically investigate PFC lateralization in iRISA in addiction are needed. Furthermore, there are clear gender differences in responses to drugs and in the transition to addiction, and imaging studies are increasing our understanding of the sexually dimorphic features of the human brain. However, so far, few well-controlled studies have focused on sex differences in the role of the PFC in addiction; instead, many studies use either female or male subjects (mostly males). Studies are also needed to explore the potentially modulating effects of other individual characteristics; of particular interest are the impact of co-morbid disorders (for example, depression may exacerbate deficits in addicted individuals) and of the recency of drug use and duration of abstinence (for example, cocaine may reduce or mask underlying cognitive or emotional impairments in cocaine-addicted individuals). Longitudinal studies would enable examination of these issues, which are of particular importance to those who abstain from drugs in the hope that PFC function will recover. Furthermore, comparison between different types of abused substances would allow differentiation between factors that are specific to certain drugs from factors that could be common across addiction populations. Instead of treating the heterogeneity of neural and behavioural changes in addiction as noise, studies could explore it with the goal of answering key questions: is PFC dysfunction in iRISA more prominent in certain addicted individuals than in others? Does self-medication drive drug taking more in some individuals than in others? How does co-morbid drug use, which is more the rule than the exception (for example, most alcoholics are nicotine-addicted), affect the neurobiology in addiction? What is the implication of this variability to treatment outcome and recovery? Most importantly, how can we use these laboratory results on the PFC functioning in addiction to inform the design of effective treatment interventions?

Summary and conclusions
In general, neuroimaging studies have revealed an emerging pattern of generalized PFC dysfunction in drug-addicted individuals that is associated with more
negative outcomes — more drug use, worse PFC-related task performance and greater likelihood of relapse. In drug-addicted individuals, widespread PFC activation upon taking cocaine or other drugs and upon presentation of drug-related cues is replaced by widespread PFC hyporeactivity during exposure to higher-order emotional and cognitive challenges and/or during protracted withdrawal when not stimulated. The PFC roles that are most pertinent to addiction include self-control (that is, emotion regulation and inhibitory control) to terminate actions that are not advantageous to the individual, salience attribution and maintenance of motivational arousal that is necessary to engage in goal-driven behaviours, and self-awareness. Although activity among PFC regions is highly integrated and flexible, so that any one region is involved in multiple functions, the dorsal PFC (including the dACC, DLPPC and inferior frontal gyrus) has been predominantly implicated in top-down control and meta-cognitive functions, the ventromedial PFC (including subgenual ACC and mOFC) in emotion regulation (including conditioning and assigning incentive salience to drugs and drug-related cues), and the ventrolateral PFC and lateral OFC in automatic response tendencies and impulsivity (TABLE 1). Dysfunction of these PFC regions may contribute to the development of craving, compulsive use and ‘denial’ of illness and the need for treatment — characteristic symptoms of drug addiction. This PFC dysfunction may in some instances precede drug use and confer vulnerability for developing substance use disorders (BOX 3). Irrespective of the direction of causality, the results of the neuroimaging studies that are reviewed here suggest the possibility that specific biomarkers could be targeted for intervention purposes. For review, perhaps these PFC abnormalities could be used to identify the children and adolescents who would benefit most from intensive drug abuse prevention efforts, and perhaps medications can ameliorate these deficits and help addicted individuals to engage in rehabilitation treatment.
New Findings on Biological Factors Predicting Addiction Relapse Vulnerability

Rajita Sinha

Abstract Relapse is a highly prevalent phenomenon in addiction. This paper examines the new research on identifying biological factors that contribute to addiction relapse risk. Prospective studies examining relapse risk are reviewed, and clinical, biological, and neural factors that predict relapse risk are identified. Clinical factors, patient-related factors, and subjective and behavioral measures such as depressive symptoms, stress, and drug craving all predict future relapse risk. Among biological measures, endocrine measures such as cortisol and cortisol/corticotropin (ACTH) ratio as a measure of adrenal sensitivity and serum brain-derived neurotrophic factor were also predictive of future relapse risk. Among neural measures, brain atrophy in the medial frontal regions and hyperreactivity of the anterior cingulate during withdrawal were identified as important in drug withdrawal and relapse risk. Caveats pertaining to specific drug abuse type and phase of addiction are discussed. Finally, significant implications of these findings for clinical practice are presented, with a specific focus on determining biological markers of relapse risk that may be used to identify those individuals who are most at risk of relapse in the clinic. Such markers may then be used to assess treatment response and develop specific treatments that will normalize these neural and biological sequelae so as to significantly improve relapse outcomes.

Keywords Addiction relapse · Stress dysregulation · Drug craving · Cortisol · Cortisol/ACTH ratio · Serum BDNF · Anterior cingulate · Biomarkers · Human studies · Biological factors · Vulnerability

Introduction

Addictions are among the most prevalent psychiatric disorders in the world. Nicotine smoking and excessive alcohol use are the top behavioral conditions causing high levels of global disease burden. The chronic, relapsing nature of addictive disorders is a key factor contributing to high disease burden. Although we have US Food and Drug Administration–approved treatments for nicotine, alcohol, and opioid addiction, more than two thirds of individuals are known to relapse after initiating treatment for substance use disorders. Furthermore, there are no validated biological markers to identify those at high risk of relapse. However, several new research advances in the past decade have moved the field closer to understanding the biology of relapse risk. The purpose of this paper is to describe these advances and to indicate the goals for developing indices of relapse risk that may be utilized in the clinic.

Addiction Relapse Vulnerability

It has long been known that addictive disorders are chronic and relapsing in nature [1, 2]. Recent estimates from clinical treatment studies suggest that more than two thirds of individuals relapse within weeks to months of initiating treatment [3, 4, 5]. For 1-year outcomes across alcohol, nicotine, weight, and illicit drug abuse, studies show that more than 85% of individuals relapse and return to drug use within 1 year of treatment [2]. Data from 878 patients entering a large, publicly funded, Yale University–affiliated addiction treatment facility in the New Haven, Connecticut, area acquired over a 1-year period were assessed for proportion of patients who were abstinent at discharge. Patients are classified here on the basis of their primary...
drug of abuse (e.g., alcohol, opiates, cocaine, marijuana), excluding nicotine, as described in Dodge et al. [6]. Patients participated in state-of-the-art, empirically based behavioral and pharmacologic therapies. Proportion abstinent at discharge was assessed based on most recent urine toxicology screening and patient and clinician reports. Findings indicated that less than 25% of primary marijuana- and cocaine-dependent patients were abstinent at discharge, while less than 35% were abstinent from alcohol and opiates over the course of a 1-year period (Fig. 1). The latter rates were higher, as non-agonist medications for alcohol and opioids were actively used in treatment in conjunction with behavioral relapse prevention approaches. These findings are consistent with previously reported observations on relapse rates, and suggest a critical need to understand the mechanisms that make addiction relapse likely, identify sensitive and specific biomarkers for relapse risk, and develop therapies to target relapse risk in order to improve addiction relapse outcomes.

Is There a Biology of Relapse?

Because addiction relapse is a common phenomenon, research in the past decade has focused on whether there is a biology underlying relapse susceptibility, and if so, whether it is possible to develop new treatments to decrease relapse risk [7, 8]. The most common reasons for relapse given by substance-abusing patients include stress, negative mood and anxiety, drug-related cues, temptations and boredom, and lack of positive environmental contingencies (e.g., job, family relationships, responsibilities) [9]. To understand how and why recovering addicted individuals succumb to relapse, particularly in the context of external environmental stimuli and interoceptive cues, it is important to examine the psychobiological consequences of chronic drug use and assess whether such changes are involved in increasing relapse risk.

Chronic Substance Use, Stress, and Associated Subjective and Behavioral Changes

High levels of stress and trauma exposure are commonly associated with substance use disorders [10, 11]. Increases in irritability, anxiety, emotional distress, sleep problems, dysphoria, aggressive behaviors, and drug craving are common during early abstinence from alcohol, cocaine, opiates, nicotine, and marijuana [10]. The dependent state is marked by negative affect, distress, and anhedonia during early abstinence, which relates to neuroadaptations in brain reward and stress pathways [7, 10, 12–15].

Chronic abuse of substances also results in greater incentive salience such that there is an increased “wanting” of drug, particularly in stress- and drug-related contexts [16]. Thus, acute stress exposure in the laboratory increases drug craving and anxiety in individuals dependent on opiates, alcohol, nicotine, cocaine, and marijuana [17, 18, 19, 20]. Similarly, substance abusers report significantly higher levels of drug-related and drug cue-related craving and attentional bias than healthy controls [21–24].

Prediction of Subsequent Relapse Risk

Several treatment studies have shown that higher levels of psychological withdrawal or “abstinence” symptoms, such as subjective distress, irritability, drug craving, sleep, and cognitive problems, occurring during early drug abstinence, even beyond the acute withdrawal phase, are associated with worse treatment outcomes among smokers, cocaine addicts, heroin-dependent individuals, and alcoholics [10]. In general, findings indicate that the greater the severity of dependence and of such drug abstinence symptoms, the worse the treatment outcomes will be.

Many human studies have shown that stress and trauma are associated with drug relapse [4, 7, 25–29, 30, 31, 32]. While an important strength of these studies over previous correlational studies of stress and relapse has been the prospective assessment of drug use for a follow-up period in order to predict future relapse risk, studies have varied in their assessment of stressful life events, the time period of follow-up to assess future relapse risk, and in the methods used for relapse assessment, which may have led to some negative results [33].

Prospective studies of relapse risk show that several clinical variables, such as depressive symptoms and drug craving, are predictive of subsequent relapse risk. Higher depression scores predicted shorter time to relapse and less
likelihood of abstinence [6, 34]. Higher craving levels during abstinence and in outpatient treatment are known to predict relapse and return to drug use [3, 29, 31]. Several studies have shown that exposure to certain stressors in the laboratory, including guided imagery stress scripts, Trier Social Stress Task [35], and systemic injections of the stress hormone corticotropin-releasing factor (CRF), increases subjective self-reports of drug craving [7, 17, 36–38]. These studies elegantly show the cause-and-effect relationship between stress and drug cue exposure and drug craving [7, 17]. Limited cause-and-effect evidence exists for stress exposure and relapse in laboratory studies [19••]. Other research combining the laboratory-based provocation of stress, negative affect, and drug craving with a prospective assessment of relapse in the real world have shown that stress-induced and cue-induced craving and stress and lower positive emotional responses are predictive of subsequent time to relapse and drug use outcomes [5, 18••, 39–41•]. Additionally, ecological momentary assessment (EMA) techniques have been used recently to assess daily stressors and negative affect and their association with both drug use and non–drug use events in a within-subjects case-crossover design in the real world. These studies have also shown that stress and negative affect are predictive of day-to-day drug use episodes monitored in the real world setting [29, 30••, 32, 42].

Chronic Substance Use and Biological Changes

A growing body of evidence is documenting alterations in the dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, autonomic nervous system changes, and alterations in brain dopaminergic and emotion and motivational systems of addicted individuals. Research has shown that both acute and protracted withdrawal from psychoactive substances are associated with overactivity of the CRF systems as documented in preclinical studies [13] and in clinical studies showing CRF-HPA disturbances in alcoholics and opiate-, cocaine-, and nicotine-addicted individuals [18••, 19••, 21, 22, 43–48]. In cocaine-dependent women, higher levels of daily measured morning sex hormone progesterone and plasma cortisol were found during the first month of abstinence as compared to healthy controls [49]. Autonomic and noradrenergic abnormalities also have been well-documented with overactivity of these systems during acute and protracted withdrawal from opiates, alcohol, and cocaine [48, 50–53]. These findings indicate that the CRF, and CRF-related HPA and hypothalamic-pituitary-gonadal axes and the autonomic/noradrenergic systems are dysregulated during acute withdrawal, and that mild to moderate alterations exist past acute withdrawal during protracted abstinence for at least 4 to 12 weeks. This may contribute to the abstinence symptoms (discussed in the previous section), addictive behaviors, and relapse susceptibility.

Biological Changes and Subsequent Relapse

Several studies have used a prospective design to examine whether changes in biological stress responses are predictive of future relapse. In alcoholics, blunted stress- and cue-induced cortisol responses have been associated with poor alcohol relapse outcomes [40, 46, 54, 55]. Nicotine-deprived smokers who were exposed to a series of stressors showed blunted corticotropin (ACTH), cortisol, and blood pressure responses to stress, but increased nicotine withdrawal and craving scores, and these responses were predictive of poor nicotine relapse outcomes [45].

In our research, inpatient treatment–engaged, recovering cocaine- and alcohol-dependent individuals completed research participation in experimental studies during which they were exposed to stress, drug cues, and neutral relaxing scenarios and assessed for drug craving, anxiety, and stress responses. After completion of the laboratory study, the patients were discharged from inpatient treatment and observed repeatedly for 90 days to assess relapse outcomes. For the cocaine group, in which we found altered stress responses compared with controls [21, 48], higher stress-induced ACTH and cortisol responses were not associated with time to relapse, but these responses were predictive of greater amounts of cocaine consumed during follow-up [5]. In abstinent, treatment-engaged, recovering alcohol-dependent individuals, we found higher basal ACTH levels and also blunted stress- and cue-induced ACTH and cortisol responses. Relating to the higher basal ACTH levels, we also found that individuals with high cortisol/ACTH ratios (a measure of sensitivity of the adrenal glands to release cortisol in response to the ACTH signal) were more likely to relapse quickly after discharge from inpatient treatment. In fact, high cortisol/ACTH ratios more than doubled the risk of shorter time to relapse [18••]. In a recent laboratory study modeling nicotine relapse in early abstinent nicotine-dependent individuals, stress-induced increases in cortisol were associated with shorter time to resisting smoking [19••]. Finally, in opiate-abstinent, methadone- or buprenorphine- maintained individuals, higher cortisol levels during drug cue reactivity were predictive of higher relapse outcomes [56]. Thus, across abstinent, recovering cocaine-, alcohol-, opiate-, and nicotine-dependent individuals, upregulation of the HPA axis with altered responsivity of the HPA axis has been associated with poor relapse outcomes. Thus, all of these addicted groups show both higher compulsive motivation for drug (craving), along with poor stress regulatory
responses as measured by higher cortisol levels and/or altered glucocorticoid feedback, which results in an enhanced susceptibility to subsequent addiction relapse.

Evidence from preclinical studies shows chronic drug-related central changes in brain-derived neurotrophic factor (BDNF) and other growth factors during abstinence that have been associated with reinstatement of drug seeking in animal models of relapse [8, 57, 58]. In a recent study, we found morning serum BDNF levels to be significantly higher in abstinent cocaine abusers [59••] and alcoholics compared with controls (unpublished data). Furthermore, higher serum BDNF levels were highly predictive of shorter time to cocaine relapse and higher amounts of cocaine used, as well as greater number of days of cocaine use over a 90-day follow-up period [59••].

**Human Neuroimaging Studies Documenting Chronic Substance-Related Brain Changes**

Several studies have documented lower gray matter volume in cortical, thalamic, and cerebellar brain regions in individuals with substance use disorders [60–63]. More severe gray matter deficits have been reported in relapers than in abstainers [64, 65]. Assessing volumes in specific regions of the amygdala, hippocampus, and ventral striatum in alcoholics after only 1 week of alcohol abstinence, Wrase and colleagues [66] recently reported lower amygdala volumes in those who relapsed compared with those who remained abstinent. In a comprehensive analysis using voxel-based morphometry, we examined changes in gray and white matter volume in abstinent, recovering alcoholics compared with controls and assessed whether volume changes predicted time to alcohol relapse and heavy drinking relapse [67]. We found that lower medial frontal cortical and parietal-occipital volumes in recovering alcoholics significantly predicted shorter time to alcohol relapse.

Using functional neuroimaging technology and a variety of cue induction procedures, many studies have examined brain regions associated with craving in addicted individuals. Exposure to drug cues known to increase craving increases activity in the amygdala and regions of the frontal cortex [68–70]. Gender differences also have been reported with cue-related activation in the amygdala and frontal cortex of cocaine-dependent individuals [71, 72]. Cue-induced craving for nicotine, methamphetamine, and opiates also activates regions of the prefrontal cortex, amygdala, hippocampus, insula, and ventral tegmental area [73]. We also examined brain activation during stress and exposure to neutral imagery in a functional MRI study. Although healthy controls and cocaine-dependent individuals showed similar levels of anxiety (using a verbal 10-point analogue scale) and pulse changes during stress exposure, brain response to emotional stress in paralimbic regions such as the anterior cingulate cortex, hippocampus, and parahippocampal regions was observed in healthy controls, while cocaine-dependent patients showed a striking absence of such activation [74]. In contrast, patients had increased activity in the caudate and dorsal striatum region during stress activation that was significantly associated with stress-induced cocaine craving ratings. Similarly, stress, alcohol cue, and neutral imagery exposure were assessed in social drinkers, and robust and similar activation of medial prefrontal, anterior cingulate cortex, insula, amygdala, hippocampus, and ventral and dorsal striatal regions was seen with stress and alcohol cue exposure. Alcohol cue-induced ventral and dorsal striatal activity correlated with alcohol cue-induced craving in men [75].

Recent positron emission tomography studies have also shown significant positive correlations between the dorsal striatum and drug cue-induced cocaine craving [76, 77]. These findings are consistent with the results of imaging studies, with alcoholic patients showing an increased association between dorsal striatum regions and alcohol craving in response to presentation of alcohol-related stimuli [78, 79]. Using positron emission tomography imaging with alcoholics and cocaine patients, research has shown a significant association between dopamine D2 receptor binding in the ventral striatum and drug craving, as well as motivation for self-administration [80–82].

Neuropsychological and imaging studies examining prefrontal executive functions, including impulse control, decision making, and set shifting, have shown executive function deficits and hypo-frontal responses in addicted individuals as compared with control volunteers [83–89]. Together, these data show a distinct pattern of findings indicating that increased stress- and cue-induced craving and compulsive drug-seeking states in addicted individuals are associated with greater activity in the striatum, but decreased activity in specific regions of the cingulate and prefrontal cortex and related regions involved in controlling impulses and emotions [88].

**Neural Correlates of Addiction Relapse**

In a study by Paulus et al. [90], recovering methamphetamine abusers who were studied in a decision-making task during a functional MRI session early in their recovery were then assessed 1 year after treatment to determine neural correlates of methamphetamine relapse. Findings indicated an important role for the middle frontal, posterior cingulate, and insula in predicting relapse to methamphetamine. In a preliminary study, Grussler and colleagues [78] reported that alcohol cue-induced activation in the putamen
(striatum), anterior cingulate, and medial prefrontal cortex was more pronounced in alcoholic patients who subsequently relapsed compared with those who had not. Kosten and colleagues [91] assessed drug cue-induced brain activation in recently abstinent cocaine-dependent patients prior to initiation in a double-blind, placebo-controlled, 12-week treatment trial of sertraline. Cocaine-dependent patients who relapsed showed greater activation in the sensory association cortex, motor cortex, and the posterior cingulate during exposure to cocaine-related videotapes.

In a preliminary study, we examined whether brain activity changes during exposure to stress imagery and stress-induced cocaine craving were associated with cocaine relapse outcomes in 31 treatment-engaged, abstinent, cocaine-dependent individuals (20 men and 11 women) [73]. Findings indicated that increased activity in the medial prefrontal cortex (Brodmann area [BA] 9, 10) was associated with a shorter time to cocaine relapse and with a higher number of days of cocaine use during the 90-day period. The medial prefrontal cortex (BA 10) is involved in emotional and autonomic regulation and with suppression of negative affect. The current findings extend its regulatory function and suggest that in abstinent drug abusers, activity in this region may represent a coping response (albeit maladaptive) in the face of emotional distress.

In two studies using different methods, brain regions important for nicotine withdrawal and smoking cessation were identified. Azizian et al. [92•] conducted a functional MRI of the Stroop task to assess cognitive control and reported that hyperreactivity of the right anterior cingulate cortex was associated with acute nicotine withdrawal. However, it is not known whether such hyperactivation in these regions associated with nicotine withdrawal predicted subsequent relapse in these individuals. Naqvi et al. [93] have shown that smokers with brain damage involving the insula, a region implicated in conscious urges, were more likely than smokers with brain damage not involving the insula to undergo a disruption of smoking addiction, characterized by the ability to quit smoking easily, immediately, without relapse, and without persistence of the urge to smoke. This result suggests that the insula is a critical neural substrate in the addiction to smoking.

Summary and Conclusions

The previous sections outline previous and recent findings on the growing research to identify sensitive markers of addiction relapse. There have been rapid changes in the technology available to assess neural and biological changes related to addictive disorders. For example, novel biochemicals such as serum BDNF can be measured reliably and have been shown to predict future cocaine relapse. Neuroimaging technologies are available to assess neural changes associated with chronic drug use and their impact on assessing relapse risk. Improvements also have been made in methods to assess drug craving, relapse, and drug use. For example, while early studies examined relapse status over a longer period, such as 1-year outcomes [33], it is now clearly evident that addiction relapse is a common and rapid process, and more frequent assessment of early relapse within weeks to months of treatment completion is important to identify high susceptibility to relapse. Several studies have shown relapse rates as high as 65% to 70% in the 90-day period following treatment.

Laboratory-based assessments of provoked drug craving and EMA methods of drug craving and stress have shown high accuracy for predicting lapse to drug use in the real world [5, 29, 30••, 31•, 94]. All these developments have begun to generate clinical outcome data that are sensitive to identifying future relapse.

While clinical symptoms such as depressive symptoms, history of trauma and high stress, and drug craving represent phenotypes important in identifying individuals entering treatment who may be most susceptible to relapse, exciting new data have begun to identify biological correlates of future relapse. These include high cortisol levels at baseline, at resting state, and with drug cue challenge. High levels of serum BDNF and high levels of adrenal sensitivity (cortisol/ACTH ratio) were also found to be predictive of relapse. Using neuroimaging, brain atrophy in the medial frontal brain region was found to predict alcohol relapse risk, and hyperreactivity of the anterior cingulate cortex was found to be associated with withdrawal and relapse risk. These findings suggest that there are key neural and biological changes associated with chronic alcohol and drug abuse that are also important as clinical predictors of relapse. With further validation of these measures in future research, it may be possible to identify an “endophenotype,” or biological profile of relapse risk, that can be used to assess relapse susceptibility in the clinic. Such markers could be highly useful not only in identifying individuals who are at risk of relapse and treatment failure, but to target specific treatments for those high relapse risk individuals.

In conclusion, it is well-known that addictions are chronic, relapsing illnesses, but systematic study to identify biological markers of addiction relapse risk has been rare. Clinical observations have shown high rates of relapse in treatment-seeking individuals within weeks and months of entering and completing treatment. Recent neural and biological evidence from clinical studies using prospective designs to assess relapse was examined to identify specific measures that show sensitivity in predicting relapse risk. Studies from cocaine-, alcohol-, nicotine-, and opiate-dependent individuals are reviewed to identify clinical,
biological, and neural measures that are predictive of addiction relapse. Stress, depressive symptoms, drug craving, cortisol and adrenal sensitivity, serum BDNF, medial frontal gray matter volume, and functional response in the anterior cingulate cortex were all identified as significant predictors of addiction relapse. Further validation of these measures along with identification of new measures could lead to the development of an endophenotype for relapse risk that may be used to screen and identify those most susceptible to relapse in the clinic. Such markers have the potential to be used as outcome measures to assess treatment response, and in the development of new treatments that reverse and normalize these biological responses to improve relapse outcomes in addiction.

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References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

11. • Enoch MA. The role of early life stress as a predictor for alcohol and drug dependence. Psychopharmacology (Berl). 2011;214:17–31. This is an excellent recent review documenting high rates of early trauma in addictive disorders.
18. •• Sinha R, Fox HC, Hong KI, et al. Effects of adolescent sensitivity, stress- and cue-induced craving, and anxiety on subsequent alcohol relapse and treatment outcomes. Arch Gen Psychiatr. 2011, (epub online). This is the first study to show that provoked alcohol craving and adolescent sensitivity are predictive of subsequent alcohol relapse.
30. •• Epstein DH, Willner-Reid J, Vahabzadeh M, et al. Real-time electronic diary reports of cue exposure and mood in the hours before cocaine and heroin craving and use. Arch Gen Psychiatr. 2009;66:88–94. This is the first study to use EMA to demonstrate
Drug addiction exacts an enormous medical, financial and emotional toll on society in the form of overdose and health complications, family disintegration, loss of employment and crime. The National Institute on Drug Abuse (NIDA), part of the US National Institutes of Health, estimates that the total cost of drug abuse in the United States exceeds US$600 billion annually, and it is particularly alarming to note a sharp increase in the abuse of prescription drugs and in drug abuse by teenagers (see the NIDA web site). These data substantiate the need for more research into the neuronal effects of drugs of abuse and the mechanisms of addiction, in the expectation of uncovering novel targets for treating and preventing addictive disorders.

Although most individuals are exposed to drugs of abuse, only a subset experience the loss of control over drug use and compulsion for drug seeking and taking that defines the addicted state. Entrance into this state is strongly influenced by both an individual’s genetic constitution and the psychological and social context in which drug exposure occurs3,4. Although the genetic contribution to risk for addiction is roughly 50%, the specific genes that are involved remain almost completely unknown. The addictive phenotype can persist for the length of an individual’s life, with drug craving and relapse occurring even after decades of abstinence. This persistence suggests that drugs induce long-lasting changes in the brain that underlie addiction behaviours.

The many cells of an individual organism, although they contain essentially identical complements of DNA, differentiate to form distinct tissues and organs through regulated changes in the transcriptional potential of each gene, based on environmental cues, cell-to-cell signals and other, probably random factors4. It is becoming clear that many of the same processes of gene regulation that are involved in the normal differentiation of cells and tissues during development are also engaged in the adult organism to mediate cellular adaptation to environmental stimuli5,6. The processes that are involved in the regulation of transcriptional potential are varied and highly complex, and include activation and inhibition of transcription factors, modification of chromatin and DNA structure, and induction of non-coding RNAs. Increasing evidence implicates these various mechanisms of gene regulation in the lasting changes that drugs of abuse induce in the brain, and offers novel inroads for addiction therapy.

Drug action and gene transcription

A seemingly similar syndrome of addiction can result from exposure to a wide variety of chemical substances or even rewarding activities, from cocaine to gambling to sex. One common mechanism in these various forms of addiction is thought to be activation of the brain’s reward circuitry, which centres on dopaminergic neurons in...
Enhanced drug responsiveness — on the behavioural, cellular and/or molecular levels — with repeated exposure to a constant dose.

Virtually all rewarding drugs or activities increase dopaminergic transmission from the VTA to the NAc and other target limbic regions, although they each employ partly distinct mechanisms and in some cases involve other neurotransmitter systems as well. The actions of drugs on the NAc are further complicated by the cellular heterogeneity of this brain region. Although drugs differ in their acute mechanisms of action, the common syndrome of addiction suggests that chronic activation of these distinct, acute mechanisms induces some shared molecular adaptations in brain reward regions that mediate the lasting nature of the addictive phenotype.

We, and others, have long proposed that changes in the transcriptional potential of genes — through the actions of transcription factors, chromatin modifications and non-coding RNAs — contribute substantially to many of the neuroadaptations that result from chronic exposure to drugs of abuse. We know that many mRNAs display altered expression in brain reward regions after chronic drug exposure, which suggests that transcription of individual genes is differentially regulated under these conditions. Over the past ~5 years, studies at the chromatin level have confirmed the involvement of such transcriptional mechanisms in vivo. Moreover, beyond stable changes in steady-state mRNA levels, this work has shown that the ‘inducibility’ of a gene — its ability to be induced or repressed in response to the next drug exposure or some other environmental stimulus — is also altered by chronic drug exposure, and that such gene ‘priming’ or ‘desensitization’ is mediated by stable drug-induced changes in the chromatin state around individual genes.

This transcriptional and epigenetic model of chronic drug action provides a plausible mechanism for how environmental influences during development can increase (or decrease) the risk for addiction later in life. For example, there is mounting evidence that stress during adolescence increases the risk of addiction, and that exposure to drugs in utero increases the risk in adolescence and adulthood. Long-lasting changes in gene transcription or in the potential for transcription that results from early-life stress or drug exposure — mediated at the chromatin level in the absence of genetic differences in the primary DNA sequence — might render an adult brain more vulnerable to the addictive process. As alterations in transcriptional potential can last for many years, this model also explains how relapse can occur despite decades of abstinence.
Recent studies of rodent models of addiction have provided considerable support for this hypothesis and have contributed substantially to our understanding of in vivo transcriptional and epigenetic regulation in the brain. Here, we highlight key examples of transcriptional and epigenetic mechanisms of drug action, and identify some of the novel potential targets for therapeutic intervention during the addiction process.

**Transcription factors in addiction**

The classic mechanism for the regulation of gene expression is through the actions of transcription factors: proteins that, in response to cell signalling pathways, bind to specific sequences of DNA — generally in the promoter or enhancer regions of target genes — and increase or repress the expression of these genes by respectively promoting or blocking the recruitment of the RNA polymerase II transcriptional complex. Transcription factors operate as part of large protein complexes, with their mechanisms of action eventually involving alterations in chromatin structure (see below). Although neurons contain hundreds of transcription factors, studies of adaptations induced by drugs of abuse have focused primarily on a small subset.

**ΔFOSB.** ΔFOSB\(^{14}\) is encoded by the FosB gene and shares homology with other FOS family transcription factors. It heterodimerizes with JUN family proteins to form activator protein 1 (AP1; also known as transcription factor AP1) complexes that bind to API sites in responsive genes to regulate transcription. There is some evidence from in vitro studies that ΔFOSB may also homodimerize\(^\text{15}\). Although all FOS family proteins are induced transiently by acute drug exposure, chronic administration of virtually any drug of abuse induces the long-lasting expression specifically of ΔFOSB\(^{14,15,17}\), a process that is most robust in the NAc and dorsal striatum, but is also seen in several other reward-related brain regions, including prefrontal cortex\(^\text{17}\). ΔFOSB induction in the NAc and dorsal striatum by drugs of abuse, regardless of whether the drug is investigator-administered or self-administered, occurs only in the subtype of medium spiny neuron (MSN) that expresses D1 dopamine receptors (D1-type MSNs)\(^{14}\) (M. K. Lobo, S. Zaman and E. J. N., unpublished observations). ΔFOSB is a carboxy-terminal truncation of full-length FOSB that is generated by alternative splicing; it lacks the two degron domains that are present in the full-length protein and that are conserved among all other FOS family proteins. This absence results in a fourfold increase in protein stability\(^\text{18}\). In addition, ΔFOSB is phosphorylated in vivo at serine 27 (as well as at several other sites) and this phosphorylation further stabilizes the protein by roughly tenfold, both in vitro and in vivo\(^{19,20}\). This intrinsic and regulated protein stability is a particularly interesting feature of the molecule, as it provides a molecular mechanism by which drug-induced changes in gene expression can persist for weeks after drug intake stops.

ΔFOSB has been linked directly to several addiction-related behaviours. In adult bi-transgenic mice, in which removal of doxycycline induces ΔFOSB overexpression specifically in D1-type MSNs of the NAc and dorsal striatum, such induction causes increased locomotor sensitivity to cocaine\(^\text{21}\), increased conditioned place-preference to cocaine and morphine\(^{21,22}\), and increased...
Self-administration
A form of operant conditioning
using a drug as a reward,
generally by administration
through an intravenous line
that is controlled directly by
the animal’s actions.

Medium spiny neurons
(MSNs). The main cell
population of the ventral and
dorsal striatum; these
GABAergic projection neurons
form the two main outputs of
these structures, called the
direct pathway (D1-type MSNs) and indirect pathway
(D2-type MSNs).

Degron domains
A specific amino acid sequence
that targets a protein for
degradation through
proteasomal or other
proteolytic processes.

Conditioned place-preference
A behavioural test in which
animals learn to prefer an
environment that is associated
with rewarding drug
administration. It provides an
indirect measure of drug reward.

cocaine self-administration. In addition, virus-mediated
overexpression studies show that cocaine-mediated
induction of ΔFOSB in orbitofrontal cortex, a subregion
of prefrontal cortex, mediates the ability of chronic
cocaine to induce tolerance to the cognition-disrupting
effects of acute drug exposure. Such overexpression
also enhances impulsivity during drug withdrawal,
and both of these effects further promote drug self-administration. Importantly, genetic or viral overexpression of ΔJUND — a dominant negative mutant of JUND
that antagonizes ΔFOSB and other AP1-mediated
transcriptional activity — in the NAc or orbitofrontal cortex blocks these key effects of drug exposure. This indicates that ΔFOSB is both necessary and sufficient for many of the changes that are wrought in the brain by chronic drug exposure. ΔFOSB is also induced in D1-type NAc MSNs by chronic consumption of several
natural rewards, including sucrose, high fat food, sex and
wheel running, and this promotes the consumption of
such rewards. This implicates ΔFOSB in the regulation
of natural rewards under normal conditions and,
perhaps, during pathological addictive-like states.

Progress has been made in identifying the broad
range of transcriptional targets (some activated and
some repressed) through which ΔFOSB produces these
various behavioural phenotypes in response to drug
exposure. By regulating numerous genes that are
related to dendritic spine architecture, including synaptotagmin, microtubule associated proteins, activity-regulated cytoskeleton-associated protein (ARC),
actin-related proteins, cyclin-dependent kinase 5 (CDK5) and kinesin, ΔFOSB mediates the structural

Figure 2 | Mechanisms of transcriptional and epigenetic regulation by drugs of abuse. In eukaryotic cells, DNA is
organized by wrapping around histone octamers to form nucleosomes, which are then further organized and condensed
to form chromatin (left part). Only by temporarily unravelling compacted chromatin can the DNA of a specific gene be
made accessible to the transcriptional machinery. Drugs of abuse act through synaptic targets such as reuptake
mechanisms, ion channels and neurotransmitter (NT) receptors to alter intracellular signalling cascades (right part).
This leads to the activation or inhibition of transcription factors (TFs) and of many other nuclear targets, including
histone-modifying proteins (shown by thick arrows); the detailed mechanisms involved in the synaptic regulation
of chromatin-regulatory proteins remain poorly understood. These processes ultimately result in the induction or
repression of particular genes, including those for non-coding RNAs such as microRNAs; altered expression of some of
these genes can in turn further regulate gene transcription. It is proposed that some of these drug-induced changes at
the chromatin level are extremely stable and thereby underlie the long-lasting behaviours that define addiction. CREB,
cyclic AMP-responsive element binding protein; DNMTs, DNA methyltransferases; HDACs, histone deacetylases;
HDMs, histone demethylases; HMTs, histone methyltransferases; MEF2, myocyte-specific enhancer factor 2; NF-kB, nuclear factor-kB; pol II, RNA polymerase II.
plasticity that is induced in NAc by cocaine34–36; it is both necessary and sufficient for cocaine-induced increases in the dendritic spine number of NAc MSNs47 (BOX 2). As discussed below, ΔFOSB controls the activity of several other transcriptional and epigenetic regulatory proteins, which then further influence NAc dendritic arborization. This suggests that ΔFOSB serves as one of the master control proteins that govern this structural plasticity. ΔFOSB also regulates proteins that are important for glutamatergic synaptic function and plasticity, including AMPA receptor subunits31,38 and Ca2+/calmodulin-dependent kinase II (CaMKII)33,39, which is consistent with the hypothesis that it mediates key aspects of the synaptic plasticity that is exhibited by MSNs after drug exposure44,45.

ΔFOSB is far more stable than all other transcription factors that have been linked to addiction so far. Nevertheless, drug relapse can occur after decades of abstinence, a timescale dwarfing even phosphorylated ΔFOSB’s prolonged turnover rate. It is possible that ΔFOSB remains stably linked to individual gene promoters for long periods of time or induces long-lasting changes to the chromatin structure of individual genes (see below) to influence relapse behaviour long after total cellular levels of the protein have returned to baseline. These possibilities remain to be investigated in future experiments.

**CREB.** Cyclic AMP (cAMP)-responsive element binding protein (CREB) forms homodimers that can bind to genes at cAMP-responsive elements (CREs). It primarily activates transcription after it has been phosphorylated at serine 133 (by any of several protein kinases), which allows recruitment of CREB-binding protein (CBP) that then promotes transcription (see below)46–48. The mechanism by which CREB activation represses the expression of certain genes is less well understood. Psychostimulants (for example, cocaine and amphetamine) and opiates increase CREB activity, and do so acutely as well as chronically — as measured by increased phospho-CREB (pCREB) or reporter gene activity in CRE–lacZ transgenic mice — and in multiple brain regions, including the NAc and dorsal striatum46–48. Experiments that involve the inducible overexpression of CREB or a dominant-negative mutant form of CREB, either in bi-transgenic mice or using viral vectors, have shown that CREB induction in the NAc, which occurs in both D1- and D2-type MSNs49, decreases the rewarding effects of cocaine and opiates44,45. This promotes drug self-administration, presumably through negative reinforcement46. CREB shows more complicated and varied responses to rewards or drugs of abuse other than cocaine and opiates. For example, chronic nicotine50 or ethanol51,52 administration reduces pCREB levels in the NAc but CREB activity seems to be necessary for nicotine to establish a place preference53. In addition, exposure to Δ9-tetrahydrocannabinol (THC, the active compound in marijuana) increases pCREB in the prefrontal cortex and hippocampus54, and stimuli that are associated with natural reward increase pCREB in the NAc55. Other CREB family proteins, such as inducible CAMP repressor (ICER; a product of the cAMP-responsive element modulator (CREM) gene) and activating transcription factors (ATFs), have also been implicated in the long-term actions of drugs of abuse and require further study56.

CREB activity has been directly linked to the functional activity of NAc MSNs. The electrical excitability of MSNs is increased by CREB overexpression, whereas dominant-negative CREB decreases it57. Possible differences between D1- and D2-type MSNs in this regard have not yet been explored. The observation that
Box 2 | Epigenetic regulation and dendritic spine plasticity

For changes in gene transcription and chromatin modifications to affect complex behaviours such as addiction, they must result in some functional output, such as a change in neuronal excitability (intrinsic membrane properties) or connectivity (synapse number or strength). Indeed, it is clear that nearly all drugs of abuse alter the structural connectivity of neurons in the reward circuitry, an effect that is most evident in changes in the number, shape and size of dendritic spines on medium spiny neurons (MSNs) in the nucleus accumbens (NAc) (see the figure, part a, which shows cocaine-induced increases in dendritic spine number that can be blocked by viral overexpression of G9a or ΔJUND, or mimicked by viral overexpression of ΔFOSB). These changes seem to be behaviourally relevant, as they correlate with behavioural sensitization\(^{34,35}\). However, certain conditions that increase spine density cause opposite behavioural effects\(^{60,65}\). Moreover, the nature of these changes varies with the abused substance, time of withdrawal and method of intake, even within a single brain region. For example, experimenter-administered cocaine increases the number of thin spines on NAc MSNs during and shortly after chronic exposure, but increases mushroom spines and dendritic complexity during withdrawal\(^{34,35}\). Resolving this discrepancy is an important future research goal. It is also likely that structural plasticity of the NAc plays a part in volition and decision-making, as self-administered drugs generally cause larger changes in spine density than the same doses administered by experimenters\(^{34,35}\). Although the molecular underpinnings of these structural changes remain incompletely understood, several factors that control gene transcription and chromatin regulation have been implicated (see the figure, part b). These include ΔFOSB\(^2\), cyclic AMP-responsive element binding protein (CREB)\(^1\), myocyte-specific enhancer factor 2 (MEF2)\(^1\), G9a\(^2\) and DNA methyltransferase 3A (DNMT3A)\(^\text{59}\), each of which has been linked directly to cocaine regulation of NAc MSN spine density. A key goal is to now identify how these epigenetic factors control cytoskeletal and cytoskeleton-altering genes to regulate spine morphology and consequently changes in neuronal circuitry and addiction-related behaviours. LIMK, LIM domain kinase; RAC, Ras-related C3 botulinum toxin substrate. Part a, right parts are reproduced, with permission, from REF. 37 © (2010) American Association for the Advancement of Science. Part a, left part is reproduced, with permission, from REF. 34 © (2010) Cell Press.

NF-κB. Nuclear factor-κB (NF-κB), a transcription factor that is rapidly activated by diverse stimuli, was studied initially for its role in inflammation and immune responses, and more recently has been linked to synaptic plasticity and memory\(^6\). NF-κB has been shown to be induced in the NAc by repeated cocaine administration, where it is required for the cocaine-induced increase in NAc MSN dendritic spine density (BOX 2) and sensitization to the rewarding effects of the drug\(^6\). It has also been associated with nicotine dependence in humans\(^6\).

A major goal of current research is to identify the target genes through which NF-κB causes cellular and behavioural plasticity. Interestingly, cocaine-induced expression of NF-κB is mediated through ΔFOSB\(^4\), illustrating the complex transcriptional cascades that are involved in drug action. The role of NF-κB in MSN spinogenesis has recently been extended to stress and depression models\(^9\). This finding is of particular importance considering the co-morbidity of depression and addiction, and the well-studied phenomenon of stress-induced relapse to drug abuse.

MEF2. Multiple myocyte-specific enhancer factor 2 (MEF2) proteins are expressed in the brain (including in NAc MSNs), where they form homodimers and heterodimers that can activate or repress gene transcription depending on the nature of the proteins that they recruit (for example, co-activator p300 and co-repressors known as class II histone deacetylases (HDACs) (see below)). Recent work suggests that chronic cocaine exposure suppresses striatal MEF2 activity, partly through D1 receptor–cAMP-dependent inhibition of calcineurin, a Ca\(^++\)-dependent protein phosphatase\(^4\). Cocaine-mediated induction of CdK5, which is a target gene for ΔFOSB\(^5\), may also be involved. This reduction in MEF2 activity is required for the cocaine-induced increase in MSN dendritic spine numbers, but seems to inhibit behavioural sensitization to cocaine\(^6\). Although these data suggest that MEF2 plays an important part in the structural and behavioural changes that result from repeated cocaine administration, they also demonstrate an apparent inconsistency between MSN spine increases and behavioural sensitization to cocaine that merits further study\(^6\). Although ethanol has been shown virus-mediated overexpression of a K\(^+\) channel subunit in the NAc, which decreases MSN excitability, enhances locomotor responses to cocaine suggests that CREB might act as a break on behavioural sensitization to cocaine by upregulating MSN excitability\(^6\). Numerous CREB target genes that mediate these and other effects on NAc MSNs have been identified\(^34,35,42,44,55\). Prominent examples include the opioid peptide dynorphin, which feeds back and suppresses dopaminergic signalling to the NAc\(^41,44\), as well as certain ion channels and glutamate receptor subunits that control NAc excitability\(^34,55\). It is interesting to compare these effects of CREB in the NAc to similar data from the locus coeruleus, where CREB has also been found to increase neuronal excitability and thereby mediate aspects of drug tolerance and dependence (BOX 3).
to decrease MEF2 expression in rat cardiomyocytes, little is known about the effects of other drugs of abuse on MEF2 function in the brain.

**Additional transcription factors.** The transcription factors that are listed here are the ones that are most extensively studied in addiction models. However, increasing evidence links several other transcription factors to drug exposure. These include the glucocorticoid receptor, nucleus accumbens 1 transcription factor (NAC1), early growth response factors (EGRs) and signal transducers and activators of transcription (STATs). For example, glucocorticoid receptor expression is required in dopamine receptor-expressing neurons to facilitate cocaine seeking but not for molecular and behavioural responses to morphine, and polymorphisms of this gene may be associated with the initiation of alcohol abuse in teenagers.

**Epigenetics of addiction**

Over the past decade, research into the regulation of transcriptional potential through modification of DNA and chromatin structure has exploded. As it became clear that epigenetic change underlies adaptations in the adult organism, investigations of epigenetic mechanisms have proven fruitful in numerous fields, including drug addiction. Here, we describe three major mechanisms of epigenetic regulation — histone tail modification, DNA methylation and microRNAs — and summarize the major findings that have linked each of these mechanisms to addiction.

**Histone tail modification.** Most DNA in eukaryotic cells is densely packed into chromatin, where 147 base pairs (bp) are wrapped around a nucleosome core in ~1.7 superhelical turns. Nucleosomes are composed of octamers that contain four histone homodimers, one of each histones H2A, H2B, H3 and H4, with H1 binding to spans of non-nucleosomal DNA. Numerous types of post-translational modifications of the amino-terminal tails of histones alter chromatin compaction to create more 'open' states (euchromatin, which is transcriptionally permissive) versus 'closed' states (heterochromatin, which is transcriptionally repressive).

Many residues in the tails of histones are covalently modified in numerous ways, resulting in a complex 'code' that is thought to control the accessibility of individual genes to the transcriptional machinery. Histone acetylation, which negates the positive charge of lysine residues in the histone tail, is associated with transcriptional activation. This process is controlled by histone acetyltransferases (HATs) and HDACs, each of which comprise multiple enzyme classes whose expression and activity are exquisitely regulated. Histone methylation has been associated with both transcriptional activation and repression, depending on the particular residue and the extent of methylation; both lysine and arginine residues can be methylated by several families of histone methyltransferases (HMTs), and this reaction can be reversed by equally diverse histone demethylases (HDMs). Histone tail modifications also include phosphorylation, ubiquitylation, sumoylation and ADP ribosylation, among many others. The prospect of deciphering the histone code is daunting, given the seemingly infinite number of possible patterns of histone modifications, and the possibility that a particular pattern may have various meanings, depending on the individual gene involved. Nevertheless, new tools are accelerating progress in mapping the epigenetic state of individual gene promoters and

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**Box 3 | Chronic morphine action in the locus coeruleus**

The locus coeruleus is the major noradrenergic nucleus in the brain, and it has served as a useful model of opiate action. Acute morphine decreases the firing rate of locus coeruleus neurons, whereas chronic exposure to the drug allows the rate to return to baseline (a phenomenon known as tolerance) and withdrawal from morphine causes firing rates to increase dramatically over baseline (a phenomenon that is characteristic of dependence and withdrawal) (see the figure, left part). Chronic morphine exerts these effects on firing rate partly by upregulating the cyclic AMP–CREB responsive element binding protein (CREB) pathway, including induction of adenyl cyclase type 8 (AC8) and CREB itself (see the figure, right part, shown by arrows in red boxes). Indeed, inhibiting or removing components of this pathway prevents the effect of chronic morphine on locus coeruleus neuron firing (see the figure, left part). As this pathway is acutely inhibited by the drug, cAMP–CREB upregulation can be seen as a classic negative feedback mechanism. These cellular and molecular effects of chronic morphine are independent of synaptic inputs and can be induced by direct activation of opioid receptors on locus coeruleus neurons in brain slices. Moreover, the proposed role for CREB in locus coeruleus, which was based originally on overexpression systems, has been validated more recently by the local knockout of endogenous CREB from locus coeruleus neurons. The nature of the ion channel (or channels) that mediate the cAMP–CREB-dependent changes in locus coeruleus excitability remains unknown, but activation of the cAMP–CREB pathway in locus coeruleus neurons is behaviourally relevant, in that it contributes to symptoms of physical opiate dependence and withdrawal, which are mediated in part by locus coeruleus activation. These studies establish the molecular details of a transcriptional mechanism of intrinsic homeostatic plasticity that is involved in the development of opiate tolerance and dependence, and have provided key insight into the chronic actions of opiates and of other drugs of abuse in several other CNS regions, including those directly related to reward, such as the nucleus accumbens and ventral tegmental area.

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**Histone methylation.** Histone methylation has been associated with both transcriptional activation and repression, depending on the particular residue and the extent of methylation; both lysine and arginine residues can be methylated by several families of histone methyltransferases (HMTs), and this reaction can be reversed by equally diverse histone demethylases (HDMs). Histone tail modifications also include phosphorylation, ubiquitylation, sumoylation and ADP ribosylation, among many others. The prospect of deciphering the histone code is daunting, given the seemingly infinite number of possible patterns of histone modifications, and the possibility that a particular pattern may have various meanings, depending on the individual gene involved. Nevertheless, new tools are accelerating progress in mapping the epigenetic state of individual gene promoters and
the genome as a whole, and future research will determine the feasibility of identifying functionally meaningful chromatin codes.42

Multiple drugs of abuse induce changes in histone acetylation in the brain, and evidence has begun to accumulate that these modifications underlie some of the functional abnormalities found in addiction models.66-70. First, global (that is, total cellular) levels of H3 and H4 acetylation are increased in the NAc after acute or chronic exposure to cocaine65,71, and gene promoters that show increased H3 or H4 acetylation have been mapped genome-wide.42 Despite these global increases, many genes show decreased histone acetylation after chronic cocaine, raising a key question as to what governs gene-specific acetylation changes in the face of global modifications. Another key question concerns the precise intracellular signalling cascades through which cocaine induces changes in histone acetylation — there is some information that such changes may be specific to D1-type MSNs and involve regulation of growth factor-associated kinases.42-47. Second, alcohol withdrawal has been shown to increase HDAC activity and reduce histone acetylation in the mouse amygdala48, and in Drosophila melanogaster the commonly abused inhalant benzyl alcohol regulates potassium channels that are tied to alcohol tolerance through H4 acetylation49. Third, exposure to THC increases HDAC3 expression in trophoblast cells50. However, this alteration was absent in a genome-wide screen of brain tissue from Δ⁹-THC-treated mice51, demonstrating that experiments on cell lines can yield effects that are very different from those found in a complex heterogeneous tissue like the brain. These data highlight the need for further research to define the effects of drugs of abuse on histone acetylation in brain in a region- and cell type-specific manner, and to identify the specific HAT and HDAC subtypes and intracellular signalling pathways that mediate this regulation in vivo.

Experimental alterations in histone acetylation potentially affect addiction-related behaviours. Short-term systemic or intra-NAc administration of nonspecific HDAC inhibitors potentiates place conditioning and locomotor responses to psychostimulants and to opiates.65,73,80. More prolonged HDAC inhibition has been reported to induce changes in the opposite direction6,48,51, perhaps through adaptations that oppose initial enzyme inhibition. Studies of specific HDAC isoforms have yielded interesting information: overexpression of HDAC4 or HDAC3 decreases behavioural responses to cocaine53,54, whereas genetic deletion of HDAC5 hyper-sensitizes mice to the chronic effects (but not to the acute effects) of the drug55. Similarly, mutant mice with reduced expression of CBP, a major HAT in the brain, exhibit decreased sensitivity to chronic cocaine56. Much additional work is needed to define the influence of specific HAT and HDAC subtypes on addiction-related phenomena.

The potential complexity involved in histone acetylation in addiction models is indicated by recent findings on sirtuins, which are considered Class III HDACs but in reality influence many non-histone proteins. Genome-wide studies of chromatin alterations in the NAc after chronic cocaine revealed an upregulation of two sirtuins, NAD-dependent deacetylase sirtuin 1 (SIRT1) and SIRT2. Pharmacological inhibition of sirtuins decreases cocaine place preference and self-administration, whereas activation increases rewarding responses to cocaine57. SIRT1 and SIRT2 induction is associated with increased H3 acetylation and increased ΔFOSB binding at their gene promoters58, which suggests that sirtuins are downstream targets of ΔFOSB. Studies are now needed to identify the proteins that are affected by cocaine-induced regulation of these sirtuins. For example, sirtuins deacetylate several transcription factors such as forkhead box O (FOXO) proteins or NF-kB, and serve scaffolding functions by contributing to transcriptional repressive complexes — processes that now warrant study in models of cocaine addiction. These findings illustrate the ability of genome-wide efforts to identify previously unknown mechanisms that are involved in drug action.

Histone methylation is also directly regulated by drugs of abuse: global levels of histone 3 lysine 9 dimethylation (H3K9me2) are reduced in the NAc after chronic cocaine exposure59, and a genome-wide screen revealed alterations in H3K9me2 binding on the promoters of numerous genes in this brain region60; both increases and decreases were observed, indicating again that epigenetic modifications at individual genes often defy global (that is, cell-wide) changes. The global decrease in H3K9me2 in the NAc is probably mediated by cocaine-induced downregulation of two HMTs, G9a and G9a-like protein (GLP), which catalyse the demethylation of H3K9me2 (Ref. 57). These adaptations mediate enhanced responsiveness to cocaine, as selective knockout or pharmacological inhibition of G9a in the NAc promotes cocaine-induced behaviours, whereas G9a overexpression has the opposite effect. Similarly, G9a downregulation mediates the ability of cocaine to increase the spine density of NAc MSNs.61,62 In additon, G9a and ΔFOSB share many of the same target genes.

Chronic cocaine also downregulates H3K9me3, a mark of heterochromatin, specifically in the NAc, and this change is associated with a decrease in the total amount of heterochromatin in NAc MSN nuclei and an increase in the volume of these nuclei63. Genome-wide mapping of H3K9me3 after chronic cocaine indicates that most of the cocaine-mediated regulation of this mark occurs on non-genic regions, including at repetitive line elements, which are consequently induced by cocaine64. Although the functional implications of this regulation are not yet known, these findings highlight the profound effects that cocaine exerts on the genome within NAc neurons.

Studies are now needed to examine the actions of other drugs of abuse on these histone endpoints, as well...
as the effect of drugs on many other types of histone modifications that are known to regulate eukaryotic gene expression in other systems, in addiction models. Examples include recent, preliminary observations of chronic cocaine-mediated regulation of histone arginine methylation and poly-ADP ribosylation, of several families of chromatin remodelling proteins, and of histone variant subunits in the NAc, all of which illustrate the complexity of epigenetic changes that are associated with drug exposure.

Moreover, it will be important to relate drug-induced modifications of histones, which occur at specific drug-regulated genes, with the recruitment of numerous additional proteins that ultimately constitute the transcriptional activation or repression complexes that mediate such regulation. For example, early studies have shown that cocaine-induced expression of CDK5 in the NAc involves a cascade of events, including binding of ΔFOSB to the Cad5 gene promoter, followed by the recruitment of CBP, increased H3 acetylation and the recruitment of specific chromatin remodelling factors, such as transcription activator BRG1 (REF. 73). Such activation also involves reduced repressive histone methylation at this promoter, which is mediated through cocaine-induced suppression of G9a. By contrast, a very different cascade mediates chronic amphetamine-induced repression of the Fos gene. Here, ΔFOSB binds to the Fos promoter and recruits HDAC1 and SIRT1, and presumably numerous other proteins. Also, chronic amphetamine induces increased repressive histone methylation at the Fos promoter, perhaps mediated through increased G9a binding. It is interesting that such increased G9a binding occurs despite the global decrease in G9a expression, once again highlighting gene-specific changes that occur on top of global modifications. Understanding the molecular basis of such gene-specific modifications — for example, why ΔFOSB triggers a cascade of transcriptional activation when it binds to one promoter, but a cascade of transcriptional repression when it binds to another — is a crucial goal of current research. So far, these efforts have been pursued on a protein-by-protein basis, which is experimentally painstaking. There is a major need in this field to develop tools to analyse the complete protein complexes that are recruited to individual genes in concert with drug exposure.

**DNA methylation.** Methylation of DNA occurs at the 5′ position of cytosine nucleotides, with the resulting methyl group projecting into the major groove of the DNA double helix. In mammals, this occurs almost exclusively in 5′-CpG-3′ sequences, and methylation is common throughout the genome — ~3% of all cytosines in human DNA are methylated — with proper cytosine methylation required for normal development, genetic imprinting and X-chromosomal inactivation.

CpG sequences are not evenly dispersed throughout the genome, but are concentrated in regions termed CpG islands. These are CG-rich regions that overlap with the promoters of 50–60% of human genes and are typically methylated to a much lower extent than CpG dinucleotides that are found outside of islands.

CpG methylation is catalysed by a family of enzymes termed DNA methyltransferases (DNMTs), some of which are responsible for the maintenance of DNA methyl states, whereas others perform de novo CpG methylation. The process of demethylation is less well understood, and may involve DNA repair mechanisms, such as growth arrest and DNA damage-inducible protein (GADD45) family members and methylcytosine dioxygenase TET1 (REFS 95–97). A variant of DNA methylation, 5-hydroxycytosine methylation, also seems to be important in gene regulation but has not yet been investigated in addiction models.

DNA methylation is generally considered to repress gene transcription through recruitment of co-repressor complexes (for example, HDACs and HMTs) that can
sterically hinder the transcriptional machinery or modify nucleosome structure. Such complexes involve several DNA methyl-binding domain proteins (MBDs)\(^2\), which are required for normal cell growth and development. Indeed, mutations in methyl CpG binding protein 2 (MeCP2), a prominent MBD, cause the majority of Rett syndrome cases and are found in a small number of patients with other autism spectrum disorders\(^1\).

There are multiple known links between DNA methylation and addiction. Cocaine self-administration increases MeCP2 expression in the NAc\(^2\) and dorsal striatum\(^2\), and lentiviral knockdown of MeCP2 in the dorsal striatum (but not the NAc) decreases drug intake under extended but not limited access conditions\(^3\).

Hypomorphic MeCP2 mutant mice show reduced locomotor sensitization and place conditioning after chronic amphetamine\(^4\), however, the same study reported that viral knockdown of MeCP2 in the NAc increases amphetamine-induced place conditioning, whereas local overexpression decreases this behavioural response\(^4\). The reasons for this discrepancy are unclear, but it seems likely that developmental abnormalities in the mutant mice, or the effects of reduced MeCP2 expression in other brain regions, explain these differences. These findings therefore emphasize the importance of using inducible and brain region-specific tools.

Two possible mechanisms for the actions of MeCP2 in drug reward have been proposed. First, a reduction in MeCP2 prevents amphetamine-mediated increases in NAc dendritic spine density while increasing the number of GABAergic synapses\(^5\). This is complemented by an increase in MeCP2 phosphorylation specifically in GABAergic interneurons in the NAc, which regulates its transcriptional activity and correlates strongly with behavioural sensitization to amphetamine\(^6\). An alternative model suggests that MeCP2 represses the transcription of specific microRNAs (see below), resulting in reduced repression of brain-derived neurotrophic factor (BDNF)\(^7\), which is also a target for CREB. BDNF has previously been described to promote cocaine self-administration\(^7\), consistent with the MeCP2 data. Although these models are not mutually exclusive, further work is necessary to integrate them with our growing understanding of the multiple brain regions and cell types that are involved in reward behaviours.

A direct link between CpG methylation and addiction involves DNA (cytosine-5')-methyltransferase 3A (DNMT3A). Repeated cocaine administration dynamically regulates DNMT3A expression in the mouse NAc, with decreases seen during early phases of withdrawal and sustained increases seen at later time points\(^8\). Experimental reduction of DNMT3A activity in the adult NAc — achieved either through virus-mediated local knockout in floxed Dnmt3a mice or through local infusion of a DNMT inhibitor — increases behavioural responses to cocaine, whereas DNMT3A overexpression in this region decreases these responses but also has the paradoxical effect of increasing NAc MSN spine density\(^9\), similar to the effects of MEF2 manipulation in this brain region\(^9\). Future research may identify the specific genes whose methylation status changes in response to chronic cocaine and consequently regulates cellular and behavioural adaptations to the drug.

These observations that chronic cocaine alters DNMT3A and MBDs in the NAc and dorsal striatum raise the possibility that drug-induced changes in DNA methylation might also occur in germ cells and be passed on to subsequent generations to regulate the propensity of the offspring for addictive behaviours. The idea of such trans-generational transmission of DNA methylation changes and the resulting behavioural plasticity remains highly speculative, although recent research has shown robust effects of adult cocaine exposure in rats on cocaine responses in their progeny\(^10\).

**Gene priming and desensitization.** Ongoing studies of chromatin regulation in addiction models support the view that epigenetic modifications at individual genes not only underlie stable changes in the steady-state levels of mRNA expression of certain genes but also alter the inducibility of many additional genes in response to some subsequent stimulus, without affecting baseline expression levels of these genes. Although such studies are still in relatively early stages of development, these types of latent epigenetic changes can be viewed as ‘molecular scars’ that dramatically alter an individual’s adaptability and contribute importantly to the addicted state.

Such priming and desensitization of genes is evident in a recently published microarray study\(^11\). Numerous desensitized genes were identified: ~10% of genes whose transcription is induced acutely in the NAc by cocaine are no longer induced by a cocaine challenge after prior chronic exposure to the drug (FIG. 3a). Conversely, numerous genes are primed: genes that are not affected by acute cocaine become inducible after a chronic course of cocaine, with approximately three times more genes being induced in cocaine-experienced animals. The mechanisms that underlie such gene desensitization and priming remain incompletely understood, our hypothesis is that epigenetic mechanisms are crucial (FIG. 3b). A subset of primed genes in the NAc show reduced binding of G9a and H3K9me2 at their promoters, suggesting the involvement of this epigenetic mark\(^12\). Desensitization of the Fos gene in the NAc, discussed above and shown in FIG. 4, involves stable increases in the binding of AFOSB, G9a and related co-repressors, which — although not affecting steady-state levels of Fos mRNA — dramatically repress its inducibility by subsequent drug exposure\(^12\).

There is now a major need in this field to investigate the many additional chromatin mechanisms that are recruited by drug exposure to mediate gene priming and desensitization, and to understand the detailed mechanisms that target those particular genes. The goal of such studies would be to identify ‘chromatin signatures’ that underlie such long-lasting regulation. The prominance of gene priming and desensitization indicates that studies of steady-state mRNA levels per se would miss important aspects of drug regulation that

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Hypomorphic

A mutation that causes a wild-type gene product to be produced at a reduced level.

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are not captured at the particular time point examined. For example, the aforementioned microarray study measured mRNA levels 1 hour after a cocaine challenge, and preliminary evidence suggests that a partially distinct set of genes show evidence of priming and desensitization at 4 hours. These observations highlight the unique utility of genome-wide assays of chromatin regulation, as such assays would reveal priming and desensitization more globally.

MicroRNAs. Increasing attention has focused on a variety of non-coding RNAs that are important in biological regulation. These include microRNAs, which are generally around 22 bp long, are found in all mammalian cells and are post-translational regulators that bind to complementary sequences on target mRNAs to repress translation and thus silence gene expression. Like histone modifications and DNA methylation, expression of microRNAs can alter the transcriptional potential of a gene in the absence of any change to the DNA sequence, and thus can be considered an epigenetic phenomenon.

Several recent studies have implicated microRNAs in addiction behaviours, and microRNAs whose expression is altered by drugs of abuse have been shown to regulate the expression of many proteins that are strongly linked to addiction.

Cocaine self-administration in rats reportedly increases expression of the microRNA miR-212 in striatum, and experimentally increasing miR-212 levels in this region decreases cocaine reward. The actions of miR-212 depend on upregulation of CREB, which is known to decrease the rewarding effects of cocaine (see above), and more recent work shows that MeCP2 may interact homeostatically with miR-212 to control BDNF expression and cocaine intake. It has been proposed that this CREB–miR-212–MeCP2–BDNF mechanism is at least partially responsible for cocaine tolerance and escalating intake. Chronic cocaine also regulates miR-124 and miR-181a in brain, where they are decreased and increased, respectively. miR-124 overexpression in the NAc reduces cocaine place conditioning, whereas overexpression of miR-181a has the opposite effect, suggesting that drug-induced regulation of these microRNAs may also act as a mechanism of tolerance and escalating intake. Like miR-212, miR-124 and miR-181a may operate through the CREB–BDNF pathway, as miR-124 overexpression downregulates both of these genes. However, these microRNAs have also been shown to affect the expression of the dopamine transporter, so their mechanisms of action are likely to be complex. Finally, argonaut 2 protein (AGO2) — which is important in microRNA-mediated gene silencing — has recently been implicated, along with several specific microRNAs, in cocaine-mediated regulation of gene expression selectively in the D2 subclass of striatal MSNs.

Other drugs of abuse have also been linked to microRNAs. Opioid receptor activation downregulates miR-190 in cultured rat hippocampal neurons in a beta arrestin 2-dependent manner, and the let-7 family of microRNA precursors is upregulated by chronic morphine exposure in mice. Interestingly, the mu opioid receptor is itself a direct target for let-7, and the resulting repression of the receptor has been suggested as a novel mechanism for opiate tolerance. In zebrafish and in cultured immature rat neurons, morphine decreases miR-133b expression, and this might influence dopamine neuron differentiation.

In addition, both acute and chronic alcohol exposure upregulates miR-9 in cultured striatal neurons, and this may contribute to alcohol tolerance through regulation of long-conductance Ca2+ activated K+ (BK) channels. miR-9 seems to preferentially downregulate BK channel isoforms that are sensitive to alcohol potentiation, perhaps shifting BK channel expression towards more tolerant subtypes. miR-9 also targets the D2 dopamine receptor and so probably influences alcohol reward.

In the future, next-generation sequencing of microRNAs in several brain regions after exposure to drugs of abuse will be essential to uncover how specific microRNAs (and, eventually, the genes that they control) are regulated. Indeed, this process has already begun, as such screens are revealing that numerous microRNAs are regulated in the NAc by chronic cocaine. For example, cocaine-mediated regulation of the miR-8 family suggests novel mechanisms for drug-induced alterations in the neuronal cytoskeletal and synaptic structure. Exploring this mechanism in drug-induced regulation of NAc dendritic morphology is an important line of future investigation.

Future directions. This Review has summarized the increasing array of findings that support a role for regulation of the transcriptional potential of myriad genes in the brain’s maladaptations to drugs of abuse. The mechanisms of transcriptional and epigenetic regulation are themselves varied and highly complex, and future studies are needed to catalogue the vast number of regulatory events that occur as well as to understand the precise underlying mechanisms that are involved. One key question is what controls the recruitment or expulsion of individual transcriptional regulatory proteins to a particular target gene. Our hypothesis is that the underlying epigenetic state of that gene is a crucial determining factor. However, if this is the case, what controls the formation and maintenance of distinct epigenetic states at particular genes? Also, what are the intracellular signalling cascades that transduce the initial drug action at the neurotransmitter-receptor level to the neuronal nucleus to regulate the epigenetic state of specific subsets of genes?

The existing literature on transcriptional and epigenetic mechanisms of addiction is limited in several key ways. So far, most studies have employed conditioned place-preference and locomotor sensitization paradigms. Although these behavioural assays provide useful insight into an animal’s sensitivity to the actions of drugs of abuse on the brain’s reward circuitry, they do not provide direct measures of drug reinforcement or addiction per se. Instead, the field needs to make greater use of drug self-administration and relapse...
Addiction research has historically neglected female subjects, particularly in animal studies, although both human and animal studies have found robust sex differences in drug responses\(^{141,144}\). In self-administration studies with various drugs, female rats are more responsive in general and exhibit particularly enhanced responses in the transition phases of acquisition or relapse compared to the maintenance phase\(^ {145,146}\). In addition, the locomotor effects of many psychostimulants are greater in female rats\(^ {147,148} \). Although, in general, ovariectomy reduces these differences and oestrogen administration increases them, this is not true of all drugs of abuse, and some contradictory results have been reported\(^ {141} \). These data suggest that drugs of abuse have differential effects on the two sexes, and that the reward system may be different between men and women; clinical evidence supports these hypotheses. Women usually have a later age of onset for substance abuse, although they progress to addiction more rapidly than men\(^ {146} \). In the specific case of cocaine, women report shorter periods of abstinence, have greater drug intake and respond more strongly to cue-induced craving\(^ {149} \). These differences may be directly related to the brain’s reward circuitry, as men have been reported to show greater striatal dopamine release than women in response to psychostimulant challenges\(^ {149} \). Interestingly, stress upregulates the expression of DNA methyltransferases (DNMTs) and DNA methyl-binding domain proteins (MBDs) in the nucleus accumbens (NAc)\(^ {150} \), these effects predominate in females and inhibition of DNMT3A in the NAc of female rats increases natural reward\(^ {151} \), suggesting that the sexes may undergo differential epigenetic regulation of the reward circuitry. Furthermore, as activation of the reward circuitry by sexual behaviour induces ΔFOSB\(^ {152,153} \) and other regulators of transcription, there is little doubt that future studies will reveal further sexual dimorphism in the regulation of transcriptional and epigenetic mechanisms by drugs of abuse — findings that may have important consequences for treatment.

Box 4 | Sex differences in drug addiction: epigenetic mechanisms?

Addiction research has historically neglected female subjects, particularly in animal studies, although both human and animal studies have found robust sex differences in drug responses\(^ {141,144} \). In self-administration studies with various drugs, female rats are more responsive in general and exhibit particularly enhanced responses in the transition phases of acquisition or relapse compared to the maintenance phase\(^ {145,146} \). In addition, the locomotor effects of many psychostimulants are greater in female rats\(^ {147,148} \). Although, in general, ovariectomy reduces these differences and oestrogen administration increases them, this is not true of all drugs of abuse, and some contradictory results have been reported\(^ {141} \). These data suggest that drugs of abuse have differential effects on the two sexes, and that the reward system may be different between men and women; clinical evidence supports these hypotheses. Women usually have a later age of onset for substance abuse, although they progress to addiction more rapidly than men\(^ {146} \). In the specific case of cocaine, women report shorter periods of abstinence, have greater drug intake and respond more strongly to cue-induced craving\(^ {149} \). These differences may be directly related to the brain’s reward circuitry, as men have been reported to show greater striatal dopamine release than women in response to psychostimulant challenges\(^ {149} \). Interestingly, stress upregulates the expression of DNA methyltransferases (DNMTs) and DNA methyl-binding domain proteins (MBDs) in the nucleus accumbens (NAc)\(^ {150} \), these effects predominate in females and inhibition of DNMT3A in the NAc of female rats increases natural reward\(^ {151} \), suggesting that the sexes may undergo differential epigenetic regulation of the reward circuitry. Furthermore, as activation of the reward circuitry by sexual behaviour induces ΔFOSB\(^ {152,153} \) and other regulators of transcription, there is little doubt that future studies will reveal further sexual dimorphism in the regulation of transcriptional and epigenetic mechanisms by drugs of abuse — findings that may have important consequences for treatment.

Assays, which are considered the best available animal models of addiction\(^ {121–123} \). Similarly, in most studies the drugs of abuse were experimenter-administered, but we know that drugs exert some distinct actions when self-administered or given within a particular environmental context. Studies that move beyond the relatively short time frames of most current experiments are also needed to examine transcriptional and epigenetic endpoints after much longer periods of drug exposure and longer periods of withdrawal from drug exposure. Such studies might lead to a molecular hypothesis that explains the phenomenon of relapse in human addicts after years or even decades of abstinence. In addition, studies should be extended from investigating cocaine action in NAc, which has been the main focus so far, to investigating several other drugs and several other reward-related brain regions. Future studies of gene regulation will better inform drug discovery efforts as they increasingly incorporate experimental paradigms that better model human addiction.

Another limitation of the existing literature is the reliance of many studies on overexpression systems — viral or transgenic — which often induce levels of expression that are far greater than those seen under normal conditions or even after drug treatment. Such overexpression of transcription factors, chromatin-regulatory proteins or their dominant-negative mutants, can lead to non-physiological changes in gene expression and subsequent alterations in cell morphology, physiology and/or behaviour. It is reassuring that many of the phenomena described above, resulting from studies that utilized overexpression systems, have been validated with other methods. For example, the genes that are regulated by overexpression of ΔFOSB in the NAc of inducible bi-transgenic mice\(^ {147} \) overlap extensively with genes that show enrichment of endogenous ΔFOSB binding after cocaine exposure\(^ {124} \). Similar caveats exist for the use of constitutive knockout animals, in which loss of a gene in early development and in all tissues makes it difficult to interpret any changes that are observed in drug regulation involving a single brain region of an adult animal. Ultimately, a truly accurate understanding of the transcriptional and epigenetic regulation of the addiction process will require the generation of novel tools that control protein expression with greater spatial, temporal and accumulation precision.

Methodological advances in epigenetics are needed as well. Current levels of experimental proof of epigenetic mechanisms of drug action have so far involved the overexpression or deletion of a given epigenetic protein (for example, an HAT, HDAC, HMT or a DNMT) within a brain region of interest. However, such manipulations affect the epigenetic states of perhaps thousands of genes without targeting those genes that are specifically altered by drug exposure. Being able to experimentally manipulate the epigenetic state of an individual gene within a discrete brain region of an adult animal would represent a major advance for the field. Tools such as artificially designed zinc-finger proteins\(^ {124} \) or sequence-specific transcription activator-like effectors (TALEs)\(^ {125} \), which are designed to bind specific DNA sequences in vivo, would offer exciting possibilities for future studies. Similarly, all genome-wide studies of drug-induced epigenetic changes in the brain so far have used total extracts of brain regions, even though we know that drugs produce very different effects on distinct neuronal and non-neuronal cell types within a given region. Genome-wide epigenetic analyses in a cell type-specific manner are crucially needed in addiction research\(^ {126} \).

Advances in bioinformatics are also needed. Genome-wide studies of transcription factor binding and chromatin modifications generate enormous datasets, which require the development of better tools to effectively mine the resulting data. For example, it will be crucial to overlay such epigenetic analyses with genome-wide changes in RNA expression and to compare data obtained from animal models with those from human post-mortem brain tissue. On a similar note, the findings from studies on drug regulation of gene expression reviewed here must be integrated with findings obtained at several other levels of analysis. How do individual differences in genome sequences relate to individual differences in epigenetic regulation? Do drug-induced epigenetic modifications occur in peripheral tissues such as blood, and do any such changes reflect addiction-relevant phenomena? Recent studies, for example, have found altered levels of methylation of the monoamine oxidase A (MAOA) and MAOB gene promoters in the blood of smokers\(^ {127,128} \). Additionally, altered methylation of MAOA in lymphoblasts is associated with nicotine and alcohol dependence in women but not in men\(^ {129} \), emphasizing the need for studies of sex differences in epigenetic regulation in addiction models, which until now have focused almost exclusively on male animals (Box 4).
As information on transcriptional and epigenetic mechanisms of addiction accumulates, it is essential to integrate it with equally important information regarding post-transcriptional (translational and post-translational) regulation to obtain a complete understanding of how chronic exposure to a drug of abuse changes the brain to cause addiction. The ultimate goal of this research is to understand basic neuronal and behavioural adaptation and, ultimately, to identify new targets for the treatment of addictive disorders and new methods for their prevention.
Neuroanatomy of Dopamine: Reward and Addiction

Katherine H. Taber, Ph.D., Deborah N. Black, M.D., Linda J. Porrino, Ph.D., Robin A. Hurley, M.D.

COVER and FIGURE 1. Approximate portions of the prefrontal cortex (PFC), important for reward, are color-coded (dorsal anterior cingulate cortex [ACC]: pink; ventral PFC: orange; orbital PFC: blue) on the left side of axial (A–C) and coronal (D–G) MRIs. Approximate extent and locations of major midbrain dopamine nuclei important for reward (dark green) and the major dopaminergic tracts (mesocortical: purple; mesostriatal: red; mesolimbic: dark blue) are color-coded on the right side of axial (A–C) and coronal (D–G) MRIs.

FIGURE 2. The cortical projections of brainstem dopamine (DA) neurons are much more extensive in humans (and other primates) than in rodents. LEFT: DA transporter mapping in human brain (colored by relative density) indicates innervation of the entire cortical mantle.1 RIGHT: DA terminal mapping in rat brain (red) indicates that innervation is largely confined to areas of the frontal, cingulate, and entorhinal cortices.2

FIGURE 3. Simplified Summary of the Reward Circuitry, Color-Coded to Match Figure 1.3,4
Abnormalities in the reward system are believed to play a role in many psychiatric disorders (for example, substance abuse, pathological gambling, major depression, schizophrenia, attention-deficit hyperactivity disorder, Parkinson’s disease, Alzheimer’s disease), so understanding the functional neuroanatomy of reward is important in neuropsychiatry. Reward is not a unitary concept. Major aspects include liking (e.g., pleasure, hedonia), wanting (e.g., motivation for reward, incentive salience), and learning (e.g., past experiences predicting future rewards). Primary (fundamental) rewards are naturally-occurring things or events that are essential for species survival and reproduction (e.g., food, sex). Secondary (higher-order) rewards are more abstract cognitive representations (e.g., monetary, artistic, altruistic, transcendent). This review will focus on the contributions of the dopamine (DA) system to reward. Many other neurotransmitter systems also participate in aspects of reward.

Reward
Research on brain areas important for reward began with the observation by Olds and Milner in the 1950s that rats will expend great effort in order to obtain electrical stimulation of multiple brain areas, including small regions within the brainstem, diencephalon, and cortex. This work was foreshadowed by earlier studies in patients with schizophrenia that focused on the septal area, in which positive immediate responses (e.g., euphoria) were reported to occur after brain stimulation.

The medial forebrain bundle in the lateral hypothalamus was a common target for electrode placement in animal studies, as stimulation in this area evoked very robust behaviors (e.g., self-stimulation to the point of physical exhaustion, willingness run across an aversive shock grid to obtain stimulation). Several lines of evidence suggested that rewarding electrical stimulation activated the dopamine (DA) projection from the ventral tegmental area (via the medial forebrain bundle) to nucleus accumbens, one part of what is now termed the ventral striatum. The ability of DA antagonists to decrease the effectiveness of rewarding electrical stimulation was particularly important. Subsequent studies indicated that the rewarding effect was not due to activation of the small, unmyelinated ascending DA fibers, but, rather, to large, myelinated fibers descending to brainstem.

Animal studies indicate that brainstem DA neurons have a baseline level of activity (tonic mode, steady activation) that enables normal downstream functioning and is modulated by both positive and negative reward-related events (phasic mode, fast activation). DA neurons increase activity in response to unexpected rewards and to stimuli that predict receipt of a reward (expectation or anticipation). During conditioned-learning, the increased activity in DA neurons shifts from the time of reward-receipt to the time of the reward-predicting stimulus. Activity is only increased by rewards if they are greater than predicted (positive prediction error). DA neurons also decrease activity when reward-expectation is not met (negative prediction error). If receipt of a reward is delayed, activity in these neurons decreases at the time the reward was expected, but did not occur, and increases when the reward is actually received. Much of behavior is guided by prediction of the future, based on past experiences. Phasic changes in DA activity, by signaling that something unexpected relating to reward has occurred, help to optimize goal-directed behavior. DA neurons also are sometimes responsive to other types of stimuli (e.g., stressful, aversive, alerting), perhaps because of their motivational salience. Functional MRI (fMRI) studies in humans have confirmed increased activation in the area of brainstem containing DA neurons during anticipation of both primary and secondary rewards.

Early studies suggested three distinct ascending DA projection systems from the brainstem (Figure 1). Originally, it was thought that the limbic and cortical projections arose from the ventral tegmental area, with the substantia nigra giving rise to the projection to sensorimotor striatum (caudate and putamen); hence, the nigrostriatal name for this tract. It is now clear that, al-
though the pathways are anatomically and functionally distinct, their cells of origin are intermixed.\textsuperscript{4,21,22}

Two of these DA pathways are particularly important for the reward system.\textsuperscript{3,4} The mesocortical DA pathway projects to multiple cortical areas and is important for many aspects of reward-processing, including hedonic evaluation, comparative valuation, and option-assessment. This pathway projects primarily to prefrontal, cingulate, and entorhinal cortices in rodents, but to the entire cortical mantle in primates (Figure 2).\textsuperscript{1,2,21,22} The mesolimbic DA pathway projects primarily to the ventral striatum, but, also, to other limbic areas (e.g., amygdala, olfactory tubercle, septum).\textsuperscript{21} This pathway is important for the positive reinforcing effects of both natural rewards and drugs of abuse.\textsuperscript{4} Ventral striatum also receives strong projections from orbitofrontal, ventral medial prefrontal, and anterior cingulate cortices, as well as limbic-related subcortical areas (Figure 3).\textsuperscript{4}

Functional imaging in humans has shown that rewards increase DA release in ventral striatum and that increasing striatal DA (by amphetamine administration) enhances rewards.\textsuperscript{4,23} Activation in ventral striatum is more strongly associated with the anticipation of reward than the actual receipt, and activation level correlates with the magnitude of the expected reward and with the effort expended to gain the reward.\textsuperscript{4,20,23} Some studies have reported decreased activation in ventral striatum when an expected reward is not received (reward prediction error).\textsuperscript{4,20} The ventral striatum contains multiple functional areas, and it is quite possible that different aspects of reward are associated with specific subregions.

Although a valuable approach, most of the studies utilizing electrical stimulation were not designed to address which aspects of reward (liking, wanting, and learning) were involved.\textsuperscript{24} The development of methods that allowed intracranial drug self-administration made it possible to more clearly identify regions participating in reward and to determine the nature of the influence (studies done primarily in rodents).\textsuperscript{6,11} Areas of the brain that endow a sensation (e.g., sweetness) with hedonic value (pleasure or liking) are generally identified by their ability to enhance liking of sensory rewards when stimulated.\textsuperscript{6} Areas presently believed to contain “hedonic hotspots” include both ventral striatum and ventral pallidum, brainstem (e.g., ventral tegmental area, parabrachial nucleus), and frontal cortex (e.g., orbitofrontal, cingulate, medial prefrontal and insular cortices). Some of these same areas are also important for endowing a sensation with motivational value (incentive-salience or wanting). The nature of the stimulation is important. Thus, there are areas within ventral striatum that evoke both liking and wanting when activated by opioids, but only wanting when activated by DA.\textsuperscript{25} Recent studies suggest that DA is very important for the motivational value of rewards.\textsuperscript{24}

Addiction

The economic costs (direct and indirect) of drug abuse are immense, estimated at $180.9 billion for the United States in 2002 alone.\textsuperscript{26} Addiction to various substances is found across cultures worldwide, and animals will voluntarily self-administer drugs-of-addiction in laboratory settings.\textsuperscript{12,13} Within the last decade, it has been recognized that behavioral addictions (e.g., gambling, pathological internet use, food) share the same core features as substance addictions. These include craving, tolerance, withdrawal, and compulsive use, despite occupational, interpersonal, and financial adversity. Although pathological levels of motivation (incentive-salience theory), learned compulsive behaviors (learning theory, habit theory), and avoidance of the negative aspects of withdrawal (negative reinforcement theory, opponent process theory) are likely all involved, the role each plays in the development and maintenance of addictions is a matter of much debate.\textsuperscript{12,23,27}

A three-stage cycle (binge/intoxication, withdrawal/negative emotional state, preoccupation/anticipation) has been proposed, in which a shift from impulsive to compulsive behaviors occurs as addiction develops.\textsuperscript{12,13} From a neurobiological standpoint, addiction is a disorder of brain reward mechanisms that are crucial for survival.\textsuperscript{9,12,13,21,28–30} Although the reinforcing value of drugs and the development of addiction involve multiple areas and neurotransmitter systems that differ by drug-of-abuse, the DA system is of central importance to all. The mesolimbic DA system is activated by all major drugs of abuse, with the ventral striatum a key structure. In animal studies, the brain stimulation required for reward (reward threshold) is reduced by acute administration of drugs of abuse. Activation in the ventral striatum is thought to be important in the reward-driven binge/intoxication stage, with engagement of dorsal striatum for the habit-formation that is believed to underlie progression to compulsive use. It has been proposed that drugs-of-abuse induce larger and more prolonged activations than natural stimuli, promoting habit-formation that is quite robust and re-
sistant to change. Intrinsically below-normal functioning in the DA system has been proposed as a risk factor for development of addiction (reward-deficiency hypothesis). Reward thresholds increase (sensitivity to rewards decrease) during protracted withdrawal after chronic drug administration, suggesting compromise of the DA system. The anhedonia and motivational deficits present during the withdrawal/negative emotional state stage may be due to decreased DA function (reward-deficiency). It has been proposed that continued drug use at this stage is more to restore a normal DA level to (“get straight”) than to evoke a large DA increase to (“get high”). Alterations in the brain’s stress systems also occur, and may be important for aversive stimulus effects and/or heightened anxiety. Altered activity in areas of prefrontal cortex and perturbations in their modulation of limbic-related subcortical areas (particularly ventral striatum, amygdala, and hippocampus) are present in the preoccupation/anticipation stage and may give rise to the deficits in executive functions (e.g., self-control, salience-attribute), and memory commonly present in addiction.

CONCLUSION

Although there is much yet be understood regarding the neurobiology of reward and its circuitry, it is certainly clear that abnormalities in these pathways can have profound effects on human behavior and on some psychiatric illnesses. As scientists are more able to map these pathways and understand the relationships with other neurotransmitters, it is anticipated that improved clinical interventions will be developed to lessen the long-term course of addiction.

References

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