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Gene expression induced by drugs of abuse

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The transition from infrequent drug use to addiction (i.e. the loss of control over consumption of a drug) probably involves changes in gene expression that restructure neural circuits in the brain. The number of genes that have been demonstrated to change expression in response to drugs has increased rapidly in recent years owing to microarray technology, which allows measurement of thousands of genes at one time. It is now important to identify which of these changes are causally related to the compulsive behavior associated with drug addiction, and which are non-specific changes related to general features of arousal or other physiological responses (e.g. stress, altered body temperature or energy metabolism).

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Abbreviations

AGS3	activator of G protein signaling 3
CART	cocaine- and amphetamine-regulated transcript
CPu	caudate-putamen
CREB	cAMP response element binding protein
DAT	dopamine transporter
EST	expressed sequence tag
IEG	immediate early gene
ip	intraperitoneal
MDM2	murine double minute clone 2
MPDZ	multiple PDZ
NAc	nucleus accumbens
NMDAR1	N-methyl-D-asparate receptor 1
PFC	prefrontal cortex
sc	subcutaneous
tPA	tissue plasminogen activator

Introduction

Drugs of abuse have powerful effects on mood and behavior. They can induce strongly positive emotional states such as euphoria (the 'high') but negative feelings such as paranoia and anxiety can also occur with acute administration of drugs of abuse. People can experience drugs of abuse without becoming 'addicted'. Thus, the acute effects are important only in so far as they contribute to the long-term chronic effects that damage mental health. When an individual takes a drug a number of times (and this number depends greatly upon the individual and the drug), a transition can occur whereby the individual begins to develop a loss of control over consumption of the drug [1]. For the purposes of this review, loss of control is defined as an inability to limit intake to healthy or socially acceptable levels. Loss of control is a critical feature of drug addiction as defined by Heidbreder and Hagan in this issue.

The molecular, cellular and physiological mechanisms that mediate the transition from occasional controlled drug use to the loss of control that, in part, defines addiction are not known. However, it is widely thought that changes in gene expression in the central nervous system (indexed by levels of mRNA) play a critical role [2]. Here, we review recent studies that document changes in gene expression in response to drugs of abuse. Most of these studies used an animal model (mice or rats) but we also include several human studies. Instead of limiting discussion to one drug, we consider several drugs including cocaine, methamphetamine, amphetamine, alcohol, morphine, marijuana and nicotine. We point out the similarities and differences in responses to the different drugs when such differences are know to occur. We also distinguish those studies that report acute (single exposure) from those investigating chronic (repeated) drug effects. Note that this review will not discuss patterns of gene expression that increase or decrease vulnerability for drug dependence (for that see Crabbe [3] and also the chapter by Mayer and Höllt in this issue). Instead, it will focus on the effects of the drugs, themselves, on gene expression.

One important confounding variable to consider when interpreting these studies is that drugs can induce changes in body temperature [4], stress axis activity [5] and/or energy metabolism, and that these physiological responses themselves can affect gene expression. Thus, it is difficult to identify which changes in gene expression are causally related to behaviors associated with drug abuse, such as excessive consumption or seeking of a drug, and which are secondary effects of these physiological responses. Few studies have explicitly attempted to deal with this problem. One strategy would be to block the change in gene expression after drug exposure (e.g. through the use of RNA interference [6]) to see whether this would prevent the drug-related behavior. If this strategy is used, it will be important to include a control group that receives the RNA interference without the drug to determine the effect of the RNA interference

itself on behavior. This is to make sure that the RNA interference specifically blocks the drug-related behavior as opposed to producing a general behavioral deficit. An alternative approach is to prevent the intermediate physiological response from occurring (e.g. adjust the ambient temperature to eliminate drug-induced changes in body temperature) to see whether the drug-induced changes in gene expression still occur. These confounding variables are important and should be addressed before it can be confidently put forward that a particular change in gene expression plays a causal role in a behavioral or psychological state relevant to drug abuse. In this review we discuss some recent studies that have taken a creative approach toward solving this problem.

Transcription factors: coordinators of gene expression

A primary mechanism by which drugs of abuse affect gene expression is through changing the concentration of transcription factors in the nuclei of cells [7]. Drugs of abuse alter neurotransmission at synapses in the brain and cause biochemical reactions (signal transduction cascades) that eventually reach the nucleus. Once in the nucleus, these signals affect gene expression; some of the first genes to change expression are transcription factors. Once translated into proteins, these factors bind to regulatory regions on DNA and thereby affect the expression of other genes. Many of these factors can form complexes with each other, producing unique DNA-binding properties [8]. Thus, by altering the expression of a limited number of transcription factors, it is possible to change the expression of a wide range of genes in a coordinated fashion [9••]. Acting serially, these factors are thought to initiate and then maintain the gene expression profile that contributes to the alteration in neural physiology, behavior and psychological state associated with drug abuse.

The immediate early genes (IEGs) are among the first to be affected. These include members of the *fos* family (*c-fos*, *fosB*, *ΔfosB*, *fra1* and *fra2*), the *jun* family (*c-jun*, *junB* and *junD*) and *zif268* (for reviews see [10,11]). Increased expression of these genes in specific brain regions, such as in the nucleus accumbens (NAc), occurs rapidly in response to acute administration of a wide range of drugs of abuse, including cocaine, morphine, nicotine and alcohol, in both mice and rats [12•,13–15]. These changes are transient, returning to baseline within 4–12 h, but they can initiate stable and long-lasting changes in expression of other genes [9••]. In addition, post-translationally modified isoforms of ΔFosB are extremely stable and have been shown to accumulate with repeated drug exposure [7]. It has been hypothesized that these long-lasting gene expression effects play a crucial role in the transition to addiction.

One transcription factor of interest that is not categorized as an IEG is Nurr1, which regulates the transcription of the gene for the dopamine transporter (DAT) protein. DAT protein is a direct target of several drugs of abuse, especially cocaine, methamphetamine and amphetamine. A recent study found that Nurr1 gene expression in the substantia nigra was substantially reduced in postmortem brain specimens of subjects that tested positive for cocaine versus drug-free subjects. The expression levels of *Nurr1* were strongly correlated with levels of DAT mRNA [16].

Drugs of abuse can also activate transcription factors through biochemical mechanisms independent of gene expression changes. An example of this is cAMP response element binding protein (CREB), whose binding affinity to DNA is affected by whether it has been phosphorylated by enzymes (kinases). Some of these enzymes (e.g. protein kinase A [17], protein kinase C [18] and calmodulin-dependent protein kinases I and II [19]) can be directly regulated by the biochemical reactions that occur when drugs of abuse enter the brain (e.g. dopamine signaling, see below). This process is dependent upon changes in calcium flux and cAMP [8].

Dopamine candidate genes

Research over the past half-century has identified several neurotransmitter systems and second messenger pathways involved in the behavioral and psychological responses to drugs of abuse [1]. The components of these systems, therefore, have been the subject of intense study. The idea behind this approach is that changes in the type or concentration of critical components will have strong effects on the system as a whole. It has many virtues, one of which is that it lends itself well to hypothesis testing. In this framework, genes that encode the critical molecules under consideration are called the candidate genes. Changes in the expression of the candidate genes, or genes that regulate their function, are of interest. In this section, we will briefly review some of the traditional candidate genes that have been the focus of investigation for many years and then discuss some of the newer candidate genes.

Dopamine is the neurotransmitter traditionally associated with the rewarding and reinforcing effects of drugs of abuse [20•]. Nearly all drugs of abuse increase the concentration of dopamine in the extracellular spaces of brain regions, such as in the prefrontal cortex (PFC), the NAc and the caudate-putamen (CPu). It is thought that this repeated dopamine signal leads to changes (neuroadaptations) that represent the compensatory physiological responses to drug exposure. Some of these neuroadaptations have been hypothesized to play a causal role in the increased motivation to consume the drug.

One such example is the altered concentration of dopamine receptors on the surface of cell membranes [20•]. When dopamine is released into extracellular spaces it

binds to these receptors, which cause signal transduction cascades. Dopamine receptors are grouped into two classes, the D1 class (D1 and D5) and the D2 class (D2, D3 and D4). Activation of these different subtypes can initiate different signaling pathways resulting in varying downstream effects on gene expression [21]. One mechanism by which drugs of abuse can regulate dopamine receptors is through changing expression of the genes for the receptors. Positron emission tomography imaging studies have consistently shown long-lasting decreases in dopamine D2-like receptors on the surfaces of membranes in the brains of drug-addicted subjects, including alcoholics [20•]. However, changes in gene expression of D2-like receptors have not been consistently demonstrated [22]. Other mechanisms, such as decreased trafficking of the dopamine receptors from the cytoplasm to the cell membrane or *vice versa*, could account for the decreased concentration of dopamine receptors in the drug-addicted subjects. The trafficking process could be regulated by changes in gene expression of proteins involved in that process. One example of this is multiple PDZ (MPDZ), the levels of gene expression of which appear to be causally related to the severity of alcohol withdrawal in mice [23••]. MPDZ is a protein involved in the trafficking of serotonin and $GABA_B$ receptors from the cytoplasm to the membrane.

Gene expression of many of the second messenger molecules that constitute the dopamine signaling pathway has also been shown to change in response to drugs of abuse, including that of G proteins [24], cAMP-dependent protein kinase [25], c-Jun N-terminal kinase 3 [26] and dopamine and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) [27]. DARPP-32 is of particular interest because its activity is affected by a wide range of neurotransmitters (in addition to dopamine) and DARPP-32's role is thought to be to integrate signals coming from a variety of neurotransmitters, neuromodulators, neuropeptides and steroid hormones [28]. In addition to these second messenger and receptor changes, expression of the gene that encodes tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines (including dopamine), is reduced after chronic self-administration of cocaine [28].

New candidate genes

Several years ago, it was discovered that a specific sequence of mRNA increased in the striatum following either cocaine or amphetamine treatment. It became known as cocaine- and amphetamine-regulated transcript (CART) [29]. The peptide produced by this transcript was subsequently identified using polymerase chain reaction followed by differential display. After its discovery, the number of studies devoted to CART subsided, but interest has recently been revived. Studies suggest that CART might play a role in feeding behavior, but whether increased expression of CART is necessary for the expression of drug-related behaviors is not known, and represents an important topic for future research. One factor that might be impeding research is that receptors for CART peptides have not yet been identified [30]. Thus, it is possible that CART plays an important role in the development of addiction, but more research is needed.

Several newer molecules are under consideration. One is the activator of G protein signaling 3 (AGS3) that regulates the activity of G proteins. G proteins are key components of dopamine, serotonin, norepinephrine, metabotropic glutamate, endocannabinoid, histamine and γ-aminobutyric acid receptor signaling. A recent study found increased gene expression of AGS3 in the PFC of male Sprague–Dawley rats after eight weeks of withdrawal from repeated cocaine self administration as compared with yoked-saline controls [31••]. The self-administration was conducted in an operant chamber where the rats pressed a lever to receive an intravenous infusion of cocaine. Each cocaine treated animal was paired with a yoked-saline control who received an infusion of saline in the same temporal pattern as that of cocaine for the paired rat. In this study, the authors took extra steps to demonstrate that the changes in AGS3 gene expression were necessary for the expression of drug-related behaviors. They blocked the increase in AGS3 expression, using an antisense oligonucleotide strategy and found that this reduced the incidence of relapse to cocaine seeking after a priming injection of cocaine. This is an example of a study that explicitly determined a causal connection between gene expression and drug-related behavior, ruling out the potential confound (described in the introduction) of secondary physiological responses.

It was recently shown that acute morphine exposure induces gene expression of tissue plasminogen activator (tPA) in several brain regions including the NAc, CPu, PFC, hippocampus and amygdala of male Wistar rats [32•]. tPA is an enzyme that degrades structural fiber-forming proteins. For example, it converts plasminogen to plasmin. It is stored in synaptic vesicles and can be released into extracellular spaces when the neuron depolarizes. Recent studies have demonstrated (as reviewed in [32•]) that tPA regulates a cascade of extracellular proteolytic activities involved in neurite outgrowth, cell migration, long-term potentiation, learning and memory, and excitotoxic cell death. Through these mechanisms it is thought to play a necessary role in the remodeling of synapses.

Another gene, mouse period 1 (*mPer1*), whose expression in the suprachiasmatic nucleus has been shown to regulate circadian rhythms, is increased by acute methamphetamine (2 mg/kg ip [intraperitoneal]) in the CPu of ddY mice [33]. The function of this gene in the CPu is not

known. It could be relevant to drug addiction, but this theory requires further evidence.

A role for glutamate systems has recently been emphasized [34]. Chronic self administration of cocaine in male Lewis rats increased expression of the gene that encodes the glutamate N-methyl-D-aspartate receptor 1 (NMDAR1) in the NAc, PFC, CPu, olfactory tubercle and piriform cortex as compared with a yoked-saline and a yoked-cocaine control [35]. The yoked-cocaine group received an equal amount of cocaine but in an unexpected and non-contingent fashion. Depending upon the brain region, *NMDAR1* expression levels were up to four times as high immediately after the last day of self-administration in the cocaine self-administering animals versus the non-contingent, yoked-cocaine group. Surprisingly, levels in the yoked-cocaine group were identical to the yoked-saline group at this time-point. The pattern changed during extinction (withdrawal) though. After one day of extinction, *NMDAR1* gene expression levels were upregulated in both contingent and non-contingent cocaine groups relative to the yoked-saline group in all five regions. Levels of *NMDAR1* gene expression in the contingent group dropped below those of the saline group after ten days of extinction in some regions (NAc, PFC and CPu), whereas they returned to baseline in the non-contingent group in these regions. A different pattern was observed for the olfactory tubercle and piriform cortex. After ten days extinction, the non-contingent group showed increased levels relative to the yoked-saline group whereas no difference occurred between the contingent and saline groups [35]. These data document a change in gene expression that is specific only when cocaine administration is contingent upon a behavior being elicited to obtain the drug (i.e. lever press).

A study with Wistar rats determined the effect of chronic ethanol on the gene expression of cannabinoid receptors [36]. These receptors are traditionally known as targets for Δ9 tetrahydrocannabinol, the major psychoactive ingredient of marijuana [37]. Three-month old male Wistar rats were either given 52 days of exposure to 10% ethanol containing 0.25% w/v saccharin with no choice of plain water, or just saccharin-water. Chronic ethanol decreased cannabinoid receptor gene expression in the CPu, ventromedial nucleus of the hypothalamus and hippocampus [36]. These data suggest that cannabinoid receptors might play a role in alcohol abuse and dependence, in addition to their role in marijuana abuse.

Finally, acute administration of morphine (10 mg/kg sc [subcutaneous]), heroin (1 mg/kg sc) or cocaine (15 mg/kg sc) increased the expression of murine double minute clone 2 (MDM2) in the hippocampus of male Sprague–Dawlay rats [38]. MDM2 is a key negative regulator of p53 (a tumor-suppressor and mediator of targeted cell death). These results suggest that the chronic use of addictive drugs activates p53-mediated cell death, and that MDM2 gene expression might increase to offset the p53 activation. Neuronal death in the brain in response to drugs of abuse might contribute to the restructuring of circuits that are necessary for the development of an addiction. Thus, MDM2, by way of its ability to modulate the effects of p53, could play a contributory role in orchestrating some of the changes that occur in the brain that lead to addiction.

Gene arrays: an exploratory approach

One relatively new tool that has been gaining popularity recently owing to advances in technology is the gene expression microarray. Thanks in a large part to the human, mouse and rat genome projects it is now possible to measure the expression of nearly every gene within the genome in one experiment. This can be done relatively quickly and easily. In contrast to the candidate gene approach discussed above, where a specific gene or genes are targeted because they are predicted to play a causal role in the drug responses, the gene microarray approach is less biased with regard to the genes that are sampled. This is because all the genes on the array are measured, which amounts to typically thousands and even up to tens of thousands of genes. The functions of many of the genes on these arrays (up to two-thirds of genes) are not known. These are named expressed sequence tags or ESTs. The rest of the genes on the array have known biological functions, at least under certain conditions.

In theory, the microarray approach is extremely powerful. However, this research is still in its infancy. Table 1 summarizes results of several recent gene-expression microarray studies. Hundreds of genes appear to change expression in response to administration of drugs of abuse. Currently, most investigators use bioinformatics tools to classify genes based on known biological function, and suggest functions for novel genes based on sequence homology, brain region and developmental patterns of expression. A microarray experiment 2–3 years ago produced a list of genes. Now, we have progressed to a state at which we are now able to produce a list of gene families. However, no clear picture of the biology has yet emerged from these data. Part of the problem is the sheer number of genes and pathways to consider. It will be important to develop a strategy to focus the number of genes to a manageable number to make sense of the biology.

Another problem is that none of these studies have controls for the effects of body temperature, stress or energy metabolism in response to the drug administration. Many of the changes reported in Table 1 might reflect non-specific physiological responses. One strategy that could be used to focus results is to use animal models to compare the gene expression profiles that result from different types of stimuli, such as shock, restraint-stress,

Table 1

Summary of several recent microarray studies.

Drug	Dose (time points for tissue sample)	Animal (brain region)	Array	Gene expression changes	Ref
Ethanol	6 g/kg ip (a near lethal dose), versus saline (6 h after injection).	Adult male C57BL/6J and DBA/2J inbred strains of mice (whole brain).	Affymetrix murine oligonucleotide GeneChips U74Av2 and U74Bv2 containing 24 000 genes and/or ESTs.	61 genes changed expression, including those known to function in oxidative stress response, cell cycle transition/ arrest apoptosis and glucose transport/metabolism. However, a limitation of this study is that the whole brain was sampled. Different genes are expressed in different brain regions, even among subnuclei within a region [50].	[51]
Ethanol	4 g/kg ip (an anesthetic dose), versus saline. Also 72 h in a chamber with ethanol vapour, versus air. (7 h after injection and 7 h after removal from ethanol vapor chamber).	Adult male C57BL/6J and DBA/2J inbred strains of mice (hippocampus).	Incyte genomics cDNA mouse GEM^{TM} array containing 7634 genes and/or ESTs.	Hundreds of genes changed expression in many categories, including those involved in cell cycle, apoptosis, signal transduction and transcription factors. Chronic withdrawal induced more changes than the acute treatment. More genes changed expression in the DBA/2J strain, which showed the greater behavioural signs of withdrawal.	[52]
Ethanol	12% (v/v) ethanol solution as the only source of liquid for 15 months, versus tap water.	Female Lewis rats four-weeks old (dorsal hippocampus).	GeneFilters GF300 cDNA microarray containing 5000 genes and/or ESTs.	Genes including those involved in oxidative stress and membrane trafficking changed expression.	[53]
Ethanol	(Post-mortem).	Human alcoholics versus non-alcoholics (frontal cortex).	Affymetrix oligonucleotide HuGeneFL array containing 4000 genes and/or ESTs.	Expression of 106 genes differed between the alcoholics and non-alcoholics. Expression of myelin-related genes, in particular, was down regulated in alcoholics.	[54]
Cocaine	(Post-mortem).	Human cocaine users versus non-users (NAc).	Affymetrix oligonucleotide microarrays U133A and U133B containing 39 000 genes.	52 genes were differentially expressed, including increased expression of cocaine- and amphetamine-regulated transcript [29]. The most robust finding was a decrease in several myelin-related genes.	[55]
Cocaine	3-hourly ip injections of 15 mg/kg, thought to represent 'binge', versus 3-hourly injections of saline. Two other groups received the binge or saline treatment for three consecutive days. (Animals were sampled 30 min after the final injection).	Adult male Fischer rats (caudate).	Affymetrix oligonucleotide microarrays rat genome U34A containing approximately 8000 genes and/or ESTs.	89 genes were upregulated and 8 downregulated following 1 day of 'binge' cocaine. Following 3 days of 'binge', 21 genes were upregulated and 17 were downregulated. Many were immediate-early genes. Other changes included increased expression of GluR2 glutamate receptor, D1 dopamine receptor and the rat period 2 (*rper2*) gene, which plays a role in circadian rhythms, at least in the suprachiasmatic nucleus. Regulator of G-protein signaling 4 (RGS4) was downregulated.	[56]
Methamphetamine	40 mg/kg, ip (a high dose) versus saline (2, 4 and 16 h after injection).	Male, outbred CD1 mice (cortex).	Clontech Mouse Atlas cDNA expression array containing 588 genes.	The early pattern was characterized by increased expression of IEGs, including *c-fos*, *c-jun*, *jun-B* and *junD*. At the later time-points, an increased number of genes related to cell death, DNA repair and DNA protection were upregulated.	[57]
Amphetamine	5 mg/kg ip (moderate dose), versus saline (1 and 3 h after injection).	Male Sprague–Dawley rats (striatum).	Atlas 1.2 cDNA expression array containing 1176 genes.	This study took the extra steps to replicate the entire experiment three times, with new animals each time. They identified large increases in expression levels of three IEGs (*Arc*, *NGF1-A* and *NGF1-B*) and demonstrated a large decrease in a RGS4.	[58]
Morphine	Series of ip injections over 10 days with increasing doses (10–50 mg/kg), versus saline (4 h after the final dose on the eleventh day).	Male eight-week old Wistar rats (frontal cortex).	Affymetrix oligonucleotide U34A array containing approximately 8000 genes and/or ESTs.	23 genes showed differential expression. A majority of these were heat-shock related genes. Also, the rat period 2 (*rper2*) gene, which plays a role in circadian rhythms, at least in the suprachiasmatic nucleus, was also significantly increased in the frontal cortex.	[59]

drugs of abuse and natural rewards. This would ultimately allow identification of gene expression changes that are specific to a drug of abuse, rather than a general physiological response to a variety of stimuli. However, this brings up another problem. Each of the studies summarized in Table 1 used a different type of microarray. Before a meaningful comparison can easily be made across studies, a standardized methodology will have to be agreed upon including a standard choice of microarray technology and data analysis.

These methodological concerns, which reflect the state of the art in a new and rapidly developing technology, make it difficult to draw overall conclusions from the data in Table 1. However, we wish to point out a few cases where consistent results were found among these studies and those investigating specific candidate genes discussed in the previous sections. Increased expression of CART was observed in the NAc of the human cocaine users (Table 1) and has also been shown to increase in the striatum in response to cocaine or amphetamine in several animal models (as reviewed in [29]). The expression of genes involved in circadian rhythms increased in the frontal cortex of rats in response to chronic morphine, in the CPu of rats in response to 'binge' cocaine administration (see Table 1) and in the CPu of mice in response to acute methamphetamine [33]. A regulator of G-protein signaling gene (*RGS4*) increased in the NAc of human cocaine users (Table 1) and a related gene (*AGS3*) in the PFC was demonstrated to play a necessary role in relapse to cocaine seeking behavior in rats [31••]. The gene for a glutamate receptor (GluR2) increased in the NAc of human cocaine users and another glutamate receptor gene (*NMDAR1*) was found to increase in the NAc of rats who chronically self-administered cocaine [35]. The hypothesis that the dopamine system plays a role in drug addiction is supported by both the microarray experiment showing that 'binge' cocaine administration in rats increases expression of the dopamine D1 receptor in the caudate (see Table 1) and the human data showing decreased *Nurr1* (which regulates expression of the dopamine transporter protein) in the substantia nigra [16]. Finally, in both the frontal cortex of the human alcoholic and the NAc of human cocaine users, myelin-related genes were down-regulated (Table 1). These changes have been postulated to contribute to the loss of white matter or brain shrinkage that results from chronic use of these drugs.

Conclusions

Many genes change expression in response to drugs of abuse. The goal now will be to sift through the data to identify which are the critical expression changes that should be targeted in preclinical trials of pharmacotherapy for addiction treatment. One strategy that might prove useful is to check whether any of the changes are specific to drugs of abuse. Many of the candidate genes whose expression has been shown to change in response to drugs of abuse also change in response to a wide range of other stimuli or emotional triggers such as stress [39,40], fear [41–43], attention and arousal [44,45], motor activity [46,47•] and aggression [48]. Even if it is shown that a change in gene expression is necessary for a drug-related behavior it is still possible that the change is general and ubiquitous over a wide range of other extreme behaviors and moods. One example is that running and cocaine both upregulate cFos and dynophin mRNA in the CPu [47•,49]. Drugs of abuse are especially strong triggers of pathological motivation (loss of control), so they must induce a specific response at some level. Studies aimed at identifying the specificity are needed. Changes in gene expression that produce long-term changes in the function of neural circuits involved in motivation are likely candidates.

The microarray exploratory approach provides a refreshing list of possible genes and gene families that might play a role in the motivational effects of drugs of abuse, although making sense of the huge number of changes has proven to be daunting. In the future it might be possible to apply this technology in a standardized fashion across several drugs, natural rewards (e.g. running, food or sex) and aversive stimuli (stress, anxiety, fear or pain) to identify the specific pathological motivational responses associated with addiction. Moreover, it will be necessary to separate those responses that are a result of intermediate physiological changes (e.g. changes in temperature or stress) from those causally related to the pathological motivation. Technology to assess this is improving and rapid progress is being made. Integration of the information across behavioral domains to identify behaviorally and/or psychologically specific gene expression profiles will be productive ventures for the future.

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This paper is important because it provides a comprehensive list of the genes that are regulated by CREB and ΔFosB, two transcription factors thought to play an important role in the development of an addiction. The authors used microarray technology combined with transgenic mice that overexpress each of these transcription factors. They also examined the gene expression profile induced by short (five days) and chronic (four weeks) cocaine administration in the mice. Importantly, the results illustrate how patterns of gene expression only partially overlap between short-term and chronic cocaine. The results support the notion that CREB and ΔFosB play wide-ranging roles in the regulation of gene expression induced by cocaine.

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A nice review of the data generated from human imaging studies. Although gene expression, *per se*, was not measured, it can be inferred that mRNA contributed to the changes discussed here. Taken together, these studies suggest that repeated exposure to drugs of abuse restructure the dopamine system in the brain, resulting in decreased dopamine release and fewer dopamine D2 receptors in the striatum. These changes in turn affect neuronal activation of brain regions such as the orbitofrontal cortex and cingulate cortex that play a role in motivation and inhibitory control.

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A remarkable set of studies demonstrating a causal connection between *Mpdz* expression levels and alcohol withdrawal severity in mice. MPDZ is a protein involved in the trafficking of serotonin and GABA receptors from the cytoplasm to the cell membrane. This is an example of a case where the potential confounding variables (described in the introduction) of the influence of secondary physiological responses (e.g. stress, body temperature or energy metabolism) on the correlation between *Mpdz* levels and withdrawal severity were eliminated through use of a genetic strategy.

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An excellent piece of work that was recognized in a preview by Ann E Kelley and Craig A Schlitz [60] in this same issue of Neuron. These data demonstrate that increased gene expression of AGS3 is necessary for the expression of cocaine seeking behavior in rats. First, the authors showed that AGS3 was upregulated in the prefrontal cortex for up to eight weeks after withdrawal from cocaine self-administration. The authors then mimicked the increase in AGS3, by fusing a functional component of AGS3 to a cell permeable peptide, and then locally injecting this construct into the prefrontal cortex. They found that this significantly increased cocaine-seeking behavior. Next, they prevented the increase in AGS3 expression using an antisense oligonucleotide strategy and found that this blocked the cocaine seeking behavior.

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These authors identify a new role for the tPA–plasmin system in regulating dopamine release. They demonstrate that tPA gene expression increases with an acute injection of morphine in Wistar rats. Then they show that tPA knockout mice have reduced morphine-induced dopamine release in the NAc, which is rescued by microinjections of tPA.

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