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Gene Expression in the Addicted Brain

Zhifeng Zhou[1], **Mary-Anne Enoch**, and **David Goldman**

Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, USA

Abstract

Addiction is due to changes in the structure and function of the brain, including neuronal networks and the cells that comprise them. Within cells, gene expression changes can track and help explain their altered function. Transcriptional changes induced by addictive agents are dynamic and divergent and range from signal pathway-specific perturbations to widespread molecular and cellular dysregulation that can be measured by “omic” methods and that can be used to identify new pathways. The molecular effects of addiction depend on timing of exposure or withdrawal, the stage of adaptation, the brain region, and the behavioral model, there being many models of addiction. However, the molecular neural adaptations across different drug exposures, conditions, and regions are to some extent shared and can reflect common actions on pathways relevant to addiction. Epigenetic studies of DNA methylation and histone modifications and studies of regulatory RNA networks have been informative for elucidating the mechanisms of transcriptional change in the addicted brain.

1. INTRODUCTION

Substance abuse and addiction to drugs and other addictive agents lead to cellular and molecular changes and are also caused in part by adaptations in epigenetic regulation and gene expression that can be measured in cells. Addictive behaviors are the outcome of allostatic maladaptation of neural circuitries (Goldman, Oroszi, & Ducci, 2005; Koob & Le Moal, 2001). Although great efforts have been made to understand the molecular basis of addiction, the mechanisms are elusive, in part because they are likely to be multiple. However, the study of gene expression in the addicted brain has already yielded valuable insights to the molecular mechanisms of maladaption. In model organisms and cellular models, several important pathway-related changes induced by acute and chronic drug exposure have been discovered. Human studies enabled by the availability of postmortem brain tissues from addicted individuals (Albertson et al., 2004; Albertson, Schmidt, Kapatos, & Bannon, 2006; Bannon, Kapatos, & Albertson, 2005; Kristiansen, Bannon, & Meador-Woodruff, 2009; Lehrmann et al., 2003; Lewohl et al., 2011; Liu, Chen, Lerner, Brackett, & Matsumoto, 2005; Mash et al., 2007; Ponomarev, Wang, Zhang, Harris, & Mayfield, 2012; Tang, Fasulo, Mash, & Hemby, 2003; Zhou, Yuan, Mash, & Goldman, 2011) have also provided critical, although somewhat divergent results for the understanding of addiction.

[1]Corresponding author: zhouz@mail.nih.gov.

Overall, there appear to be many common neuronal changes in gene expression among individuals addicted to various agents (Lehrmann et al., 2006; Marie-Claire et al., 2007; Zhou et al., 2011) and some commonalities with observations from model organisms, reflecting impact on shared molecular pathways involved in neuronal adaptation as well as drug-specific changes (Albertson et al., 2006; Celentano et al., 2009; Zhou et al., 2011).

It is clear that several differences in type of exposure alter the pattern of altered gene expression. One such factor is course of the exposure. Specific changes in early response genes and signal transduction pathways are more visible in the early stages of drug-induced neural adaptive processes (Celentano et al., 2009; Marie-Claire et al., 2007; Zhou et al., 2011), whereas prolonged exposure leads to widespread transcriptional changes of genes involved in diverse cellular functions such as ion transport, chromosome remodeling, stress and immune response, cell adhesion, cell cycle, apoptosis, protein and lipid metabolism, and mitochondrial functions (Albertson et al., 2004; Bannon et al., 2005; Mash et al., 2007; Renthal et al., 2007; Zhou et al., 2011). The impact of drug exposure on transcription is also brain region specific. In two components of the mesolimbic system, the dorsal striatum and nucleus accumbens (NAc), the expression of genes involved in dopaminergic, glutamatergic, and GABAergic transmission (Ghasemzadeh, Mueller, & Vasudevan, 2009; Hyman & Malenka, 2001; McClung et al., 2005; Schumann & Yaka, 2009) and that play key roles in drug-reward and drug-seeking behavior is strongly altered. In the hippocampus, a brain region critical for associative learning and memory, addiction alters the expression of genes involved in long-term potentiation (LTP) (Zhou et al., 2011). Genetic studies, especially ones using genomic sequencing of animal models selectively bred for addiction phenotypes, have uncovered functional variants of genes involved in neural adaptation that are directly responsible for genetic differences in the propensity to use addictive agents and in response (Zhou et al., 2013). Using “omic” approaches, it has also become possible to analyze the whole transcriptome and epigenetic patterning of the genome, and new molecular adaptive processes that contribute to addiction have recently been revealed by applying these methods both in humans and in model organisms.

2. MOLECULAR ADAPTATIONS ACCOMPANYING EARLY RESPONSE AND LONG-TERM ADAPTATIONS IN THE ADDICTED BRAIN

Knowledge of early gene expression changes in response to drug exposure has largely derived from animal studies, many of which have focused on preselected candidate genes and pathways. The molecular targets are often drug specific, for example, the dopamine transporter for cocaine and amphetamine exposure (Calipari, Ferris, Salahpour, Caron, & Jones, 2013; Peraile et al., 2010), opioid receptors and propeptide genes for opioid exposure (Diaz, Barros, Antonelli, Rubio, & Balerio, 2006), and GABA and glutamate receptors for other drug and alcohol exposure (Enoch et al., 2012; Meinhardt et al., 2013; Nona, Li, & Nobrega, 2013; Schumann & Yaka, 2009; Swanson, Baker, Carson, Worley, & Kalivas, 2001; Zhang et al., 2009; Zhou et al., 2013). Certain aspects of cell signaling, early transcriptional response, and learning have been obvious, and fruitful, targets for study in the addictions. Acute exposure to cocaine induces expression of immediate-early genes such as *Jun* and *Fos*, which encode transcription factors. The transcripts of these gene return to

control levels, and following repeated administration of the drugs, desentization is seen (Hope, Kosofsky, Hyman, & Nestler, 1992). The transcription factors FosB (Hope et al., 1992; Nestler, 2008) and CREB (Carlezon, Duman, & Nestler, 2005) have also been well documented as key components targeted by multiple signal transduction pathways and are involved in regulating expression of drug response genes. Binding of the Fos/Jun heterodimer to AP-1 sites and CREB to cAMP-response elements (CREs) in gene promoters activates transcription of the targeted genes. Another group of well-studied immediate-early gene products is the Nur transcription factors that bind to Nur-responsive elements. These are widely present in the hypothalamus–pituitary–adrenal axis and show rapid and transient increases in expression during acute exposure to addictive drugs (Campos-Melo, Galleguillos, Sanchez, Gysling, & Andres, 2013).

In the past decade, global analysis of gene expression using high-throughput microarrays and, more recently, the use of genomic sequencing, have been frequently applied to the problem of addiction (Hitzemann et al., 2013) and have shed new light on molecular pathways that are altered in the addicted brain. These studies have been conducted in diverse contexts including rodents, nonhuman primates, and postmortem human brain samples and have revealed some important divergences. The differences in what is observed appear to be mainly due to timing and exposure: each study is a snapshot of the addicted brain in dynamic processes. In rodents, some studies have profiled gene expression during drug-self administration, whereas others during withdrawal. An important distinction between the rodent models and human postmortem brain is that in rodents, “chronic exposure” usually refers to a few days or weeks, whereas in humans, it usually denotes many years of heavy use. This one fact appears to explain most of the differences observed in studies of rodents versus those on people. More widespread and divergent molecular and cellular changes have been observed in the chronically addicted human postmortem brain. In a study of postmortem prefrontal cortex from chronic cocaine abusers, Lehrmann and colleagues found expression alterations in multiple cellular functional domains, including energy metabolism, mitochondrial oxidative phosphorylation, oligodendrocyte function, cytoskeleton and related signaling, and neuronal plasticity (Lehrmann et al., 2003). Interestingly, they also noted two distinctive states of transcription regulation, an elevated gene expression profile in the recent active cocaine abusers and decreased expression state in the non-active abusers. Altered expression in cocaine addicts has also been shown in myelin-related genes. In a study by Albertson and colleagues (Albertson et al., 2004; Bannon et al., 2005) on human postmortem NAc, the most prominent changes were decreases of myelin basic protein (MBP), proteolipid protein, and myelin-associated oligodendrocyte basic protein. The expression changes were also consistent with a decrease in the number of MBP-immunoactive oligodendrocytes. A study by Mash and colleagues also found cocaine-induced expression changes in genes involved in regulating extracellular matrix integrity and angiogenesis (Mash et al., 2007). At the top of the list of affected genes was *RECK*, encoding a membrane-anchored glycoprotein serving as an inhibitor for matrix metalloproteinase-9. In addition, they also observed altered expression of genes involved in apoptosis and cell death, neurogenesis and axon guidance, signal transduction, transcriptional and translational regulation, and ion transport (Mash et al., 2007).

Our study (Zhou et al., 2011) with genomic sequencing directly examined mRNA-based transcriptome (RNA-Seq) in human postmortem hippocampal tissue from 24 men who were either cocaine addicts or alcoholics, or age-, ethnicity-, and postmortem interval-matched drug-free controls. Expression of 16,008 Refseq genes was detected. Among these, at an uncorrected $P<0.05$, we observed a total of 1994 differentially expressed genes in cocaine addicts, and 1275 differentially expressed genes in the alcoholics. After genome-wide multiple testing correction using a relatively stringent FDR cutoff of less than 0.2, there were 394 differentially expressed genes in the cocaine addicts and 48 in the alcoholics. At FDR<0.05, there were 80 differentially expressed genes in the cocaine addicts and 11 in the alcoholics (Fig. 10.1A and B).

These genes differentially expressed in the human chronic cocaine brains we studied are involved in diverse cellular functions, but there were patterns that strongly implicated certain cellular functions. Cocaine depressed the transcript levels for all five members of the BEX gene family (*BEX 1–5*), which encodes brain expressed, X-linked proteins that are thought to mediate neurotrophin signaling and neuronal differentiation (Vilar et al., 2006). There were also significant expression changes for some histone protein genes. Genes involved in regulation of transcription, gene silencing, and chromatin modification were also affected. Several of these genes had been previously implicated in cocaine addiction, including *DNMT3a*, a DNA methyltransferase which was reported to play an important role in regulating cocaine response and spine plasticity in the NAc in the rat (LaPlant et al., 2010) and *HDAC2*, a histone deacetylase found to be involved in cocaine-induced transcription changes in rat NAc and cocaine seeking behavior (Chandrasekar & Dreyer, 2010). In addition, there was also convergent evidence that chronic cocaine exposure alters expression of genes involved in RNA processing, including significant alteration in the expression of genes encoding RNA-binding and processing proteins and enrichment of differentially expressed small nucleolar (sno) RNA genes, which are involved in both ribosomal RNA and mRNA processing (Kishore & Stamm, 2006).

A particularly salient effect of long-term cocaine exposure in postmortem brains we studied was alteration in the expression of genes involved in mitochondrial inner membrane functions and oxidative phosphorylation (Zhou et al., 2011). Interestingly, these genes integral to cellular energy production have also been implicated in neurodegenerative diseases (Cho, Nakamura, & Lipton, 2010). Among the 90 genes encoding components of oxidative phosphorylation whose expression could be reliably evaluated by RNA-Seq of hippocampal mRNA, 32 were differentially expressed (uncorrected $P<0.05$), and all were downregulated. Furthermore, 74 of the 90 genes (including the 32 genes that were significantly downregulated) displayed reduced expression levels in cocaine addicts. These findings were also highly consistent with previous brain imaging studies that have revealed negative effects of cocaine on brain glucose metabolism (London et al., 1990; Lyons, Friedman, Nader, & Porrino, 1996; Macey, Rice, Freedland, Whitlow, & Porrino, 2004; Thanos, Michaelides, Benveniste, Wang, & Volkow, 2008). Furthermore, alteration of certain genes encoding for mitochondrial components induced by cocaine (Lehrmann et al., 2003) and nicotine (Wang, Kim, Donovan, Becker, & Li, 2009) exposure had also been reported previously.

3. SUBSTANCE-SPECIFIC AND SHARED GENE EXPRESSION CHANGES IN ADDICTED BRAIN

Clearly, cellular and molecular changes of neural adaptation in addiction occur in a substance-specific fashion as well as through common transduction in neurotransmission pathways. Substance-specific effects are not only due to the direct action of certain drugs of abuse on specific receptors, but also by distinctive molecular and cellular changes related to the drugs that may be related to distinctive signaling mechanisms or differences in the modulation of specific neurons and circuits. Using microarray gene expression profiling, Bannon and colleagues (Albertson et al., 2006) observed decreased expression of many genes involved in presynaptic release of neurotransmitter in the NAc of chronic heroin abusers, but not in chronic cocaine abusers. Similarly, the prominent depressed expression of myelin-related genes found in cocaine abusers was not observed in heroin abusers. Their results suggested the divergent effects of cocaine and heroin on gene expression in the NAc, despite their common effects on dopaminergic transmission. Another study by Marie-Claire et al. (2007) also reported differential effects of cocaine and 3,4-methylenedioxymethamphetamine on expression of the *Rnd* gene family involved in actin cytoskeleton regulation in mouse striatum and noted that the two drugs might act through distinctive pathways to regulate these genes.

Comparing cocaine exposure with alcohol exposure, our analysis (Zhou et al., 2011) of transcriptomes revealed a stronger shift in hippocampal mRNA expression in cocaine-addicted brains. This was manifested by both the larger number of differentially expressed genes (Fig. 10.1B) and changes in molecular and cellular functions defined by gene ontology. For example, the unidirectional depression of expression for the genes encoding for mitochondrial inner membrane and oxidative phosphorylation was only observed in the cocaine-addicted brain (Zhou et al., 2011). This strongly suggests that the inhibition is a specific effect of chronic cocaine exposure, with potential negative implications for brain energy metabolism and diverse brain functions that depend on it and with different metabolic consequences in alcoholism.

Drug-induced neuroplasticity involves some common molecular and cellular changes of the neurocircuitries (Hyman & Malenka, 2001; Kauer & Malenka, 2007; Koob & Volkow, 2010), such as dopaminergic transmission in mesolimbic system, and corticotropin-releasing factor and norepinephrine systems in the extended amygdale. We have also observed significant overlap of gene expression and pathway alteration in both cocaine- and alcohol-addicted brains. Among the 48 differentially expressed genes (FDR<0.2) in the alcoholics, 29 were common to cocaine addicts and in each case the change was in the same direction. More strikingly, for the 11 most significantly differentially expressed genes (FDR<0.05) in alcoholics, 9 were also altered to the same degree in the cocaine addicts, suggesting shared pathways impacted by both cocaine and alcohol in neuronal adaptation. It is apparent that these commonly affected protein-coding genes (Fig. 10.1C) play important roles in neuronal functions. They include *CDR1*, a cerebellar degeneration-related protein; *LRCH4*, a leucine-rich repeat-containing neuronal protein; *CACNB2*, a subunit of voltage-gated calcium channel and involved in neuronal functions; and *FAM123A* (*AMER2*), a member of the gene

family involved in neurogenesis (Comai, Boutet, Neirijnck, & Schedl, 2010). Other commonly and most significantly affected genes also encode for proteins critical in cellular functions such as histone (*HIST1H4E*), transcription regulations (*ZGPAT*, *ERF*, and *HIVEP3*) (Li et al., 2007), and mitochrondrial poly(A) polymerase (*PAPD1*). A subsequent pathway-targeted analysis of GABAergic genes also revealed common expression changes in the cocaine addicts and alcoholics from our study, such as the downregulation of *GABBR1*, *GABRG2*, and *GPHN*, a gene encoding the associated scaffolding protein gephyrin (Enoch et al., 2012).

4. REGION-SPECIFIC GENE EXPRESSION CHANGES IN ADDICTED BRAIN

It is apparent that many neuronal gene expression changes in the drug-induced adaptive process are region specific and cell specific. The mesolimbic system is critically involved in drug-reward and drug-seeking behavior and has been a focus for studies of addiction. In the dorsal striatum and NAc, medium-sized spiny neurons mediate dopaminergic, glutamatergic, and GABAergic neurotransmission (Hyman & Malenka, 2001) and rodents exposed to cocaine or during withdrawal show significant changes in dopaminergic, glutamatergic, and GABAergic neurotransmission (Ghasemzadeh et al., 2009; Hyman & Malenka, 2001; Nestler, 2001). Expression changes of genes targeted by dopaminergic and glutamatergic transmissions or genes involved in mediating transmission were also initiated during adaptation to drug exposure. Some of these genes have been relatively well analyzed such as *CART* (Douglass, McKinzie, & Couceyro, 1995), the *Fos* family (Hope et al., 1992; Nestler, 2008), *CREB* (Carlezon et al., 2005), *Arc* (Fosnaugh, Bhat, Yamagata, Worley, & Baraban, 1995), *EGR1* (O'Donovan, Tourtellotte, Millbrandt, & Baraban, 1999), *Homer-1* (Swanson et al., 2001), *MKP-1* (Ujike, Takaki, Kodama, & Kuroda, 2002), *Narp* (Hyman & Malenka, 2001), *NFκB* (Ang et al., 2001), and *CdK5* (Bibb et al., 2001). During the adaptive process, changes in the striatum take place in synergy with changes in other brain regions, particularly with changes in dopaminergic neurons in the midbrain ventral tegmental area where cocaine-induced glutamate release activates calcium–calmodulin-dependent protein kinases such as CaMKII which are involved in the process of behavioral sensitization (Fernandez-Espejo, Ramiro-Fuentes, Portavella, & Moreno-Paublete, 2008).

In our study of the hippocampal transcriptome (Zhou et al., 2011) of cocaine addicts and alcoholics, we did not observe significant expression changes for some of the genes that have previously been shown to be altered in striatum, such as *CART*, *FOSB*, *CdK5*, *NFκB*, and *HOMER*. These differences may be a manifestation of brain region-specific changes or may also be the result of stage-specific alterations in response to drug exposure because the rodent studies were performed following relatively short-term drug exposure. However, in the cocaine addicts, we did observe expression changes in genes important for hippocampal functions, such as LTP. Hippocampal functions related to short- and long-term memory processes involve synaptic plasticity, and drug-associated learning and memories are important in craving. The hippocampus also directly projects excitatory efferents to the NAc and can also activate dopaminergic neurons of the ventral tegmental area, further implicating its involvement in drug-induced changes of neural plasticity. The genes involved in LTP include specific ionotropic and metabotropic glutamatergic receptors, calcium signaling-related proteins such as calmodulin, calcium/calmodulin-dependent protein kinase, protein

phosphotase, adenylate cyclase, protein kinase A and C, mitogen-activated protein kinase, and cAMP-response element binding protein (CREB). Among these, the most significantly affected genes are the *N*-methyl D-aspartate (NMDA) receptor 2B (*GRIN2B*), a subunit of the ionotropic glutamate receptor; protein phosphatase 3 catalytic subunit α isoform (*PPP3CA*), a part of the calcium-dependent phosphatase calcineurin; and calcium/calmodulin-dependent protein kinase type II Δ chain subunit (*CAMK2D*). In addition, the list of genes relevant to LTP whose expression is altered by long-term cocaine exposure includes protein phosphatase 1 catalytic subunit β and γ isoforms (*PPP1CB* and *PPP1CC*), calmodulin 2 (*CALM2*), CREB (*CREB1*), adenylate cyclase 1 (*ADCY1*), protein kinase C β1 (*PRKCB1*), and an N-ras oncogene with intrinsic GTPase activity (*NRAS*). Although we did not observe significant changes of the LTP pathway in the alcoholics, the phosphatidylinositol signaling system, which is closely related to the LTP pathway, was significantly altered by chronic exposure to both cocaine and alcohol. These findings of gene expression changes, together with other studies that have shown the effects of cocaine on LTP (del Olmo et al., 2006; Dunwiddie, Proctor, & Tyma, 1988; Guan, Zhang, Xu, & Li, 2009; Huang, Lin, & Hsu, 2007; Smith, Browning, & Dunwiddie, 1993; Thompson, Gosnell, & Wagner, 2002; Thompson, Swant, & Wagner, 2005), provide evidence that chronic exposure to cocaine, and possibly alcohol, leads to long-term changes in the plasticity of the hippocampus and underlines the importance in addiction of molecular mechanisms for learning.

5. PERTURBATION OF THE GLUTAMATERGIC SYSTEM IN ADDICTED BRAIN

The glutamatergic system, the major excitatory system in the central nervous system, is of particular relevance to addiction through the network of interactions with dopaminergic and GABAergic transmission that underlie alcohol and drug craving and relapse. Glutamate receptors work in synergy with dopamine receptors in dendritic spines of medium-sized spiny neurons in the striatum (Cahill, Salery, Vanhoutte, & Caboche, 2014). Epi-static interactions of glutamatergic and dopaminergic genes have been claimed in alcoholics (Puls et al., 2008). Acute and chronic exposure to alcohol affects glutamate transmission (Ding, Engleman, Rodd, & McBride, 2012) and hyperfunctioning of glutamate transmission has been observed during ethanol or drug withdrawal (Hermann et al., 2012; Prior & Galduroz, 2011). Conditional knockout of the NMDA receptor GluN2B subunit in mice eliminates LTP in the bed nucleus of the stria terminalis (Wills et al., 2012) and makes the animals more sensitive to the locomotor effects of ethanol (Badanich et al., 2011). Pharmacological manipulations have demonstrated that activation of group II metabotropic glutamate receptors decreases alcohol (Rodd et al., 2006; Zhao et al., 2006) and cocaine (Jin et al., 2010) seeking and decreases alcohol-induced neurodegeneration (Cippitelli et al., 2010) in rats.

Alteration of gene expression has been linked to persistent behavioral changes in alcohol- or drug-dependent individuals in both animal and human studies (Edenberg et al., 2005; Heilig & Koob, 2007; Hwang, Stewart, Zhang, Lumeng, & Li, 2004; Liang et al., 2010; Zhou et al., 2011). In the “post-dependent” rats generated by intermittent alcohol vapor intoxication and

withdrawal, Meinhardt et al. (2013) identified a pronounced deficit of the metabotropic glutamate receptor II (mGluR2) in the pyramidal neurons of the infralimbic cortex. Among a group of glutamatergic genes that showed enriched downregulation of expression, *Grm2*, which encodes for mGluR2, was one of the genes that were most significantly affected, although the expression of *Grm3*, which encodes mGluR3, the other member of the group II metabotropic glutamate receptors, was not altered in this region. Reduction of extracellular glutamate levels in the NAc, which was readily observed in control rats upon systemic injection of mGluR2/mGluR3 agonists, was also absent in the post-dependent rats, consistent with the lack of mGluR2 function as a presynaptic receptor to downregulate glutamate release upon activation. The role of mGluR2 was further demonstrated by restoring the receptor through bilateral injection of a lentiviral vector expressing mGluR2 into infralimbic cortex. Expression of the receptor significantly reduced alcohol seeking in the post-dependent rats during the cue-induced reinstatement tests.

Using genomic sequencing, genetic linkage, functional validation, and transcriptome analysis, we found that a *Grm2* stop codon functions as a genetic determinant for alcohol preference in selectively bred alcohol preferring (P) and nonpreferring (NP) rats (Zhou et al., 2013). In contrast, genetic studies in humans to identify genes and variants underlying complex disorders and addiction have achieved only limited success, largely due to genetic heterogeneity and the limited effect size of individual loci. Animals selectively bred for alcohol and drug dependence provide potentially powerful models for the identification of genetic variants influencing addiction behaviors both because the artificial selection may collect to high frequencies variants that are rare or uncommon in the ancestral population and because of the ability to control environmental exposures and test animals under the same conditions. By exome sequencing, we uncovered a *Grm2* C407* variant from 25,715 SNPs that homozygously segregates between P and NP rats. All P rats were homozygous for this stop codon in the mGluR2 receptor ligand-binding domain, whereas none of the NP rats carried this allele. The levels of *Grm2* transcript in both striatum and hippocampus were significantly lower, and expression of mGluR2 protein was undetectable in P rats (Fig. 10.2A and B). The loss of the mGluR2 receptor was also consistent with the observation of uncompensated impairment in synaptic depression in P rats, measured as field excitatory postsynaptic potential or population spike in dentate gyrus/hippocampal and striatal slices, upon activation of the receptor by the group II mGluR agonist LY379268.

The causal role of the mGluR2 stop codon in altered alcohol preference was supported by multiple layers of evidence (Zhou et al., 2013). Genetic linkage analysis in the F2 rats derived from intercrossed inbred P and NP rats showed that homozygous stop codon carriers had significantly increased alcohol consumption and preference. Pharmacological blockade of mGluR2 receptor by mGluR2/mGluR3 antagonist LY341495 also significantly escalated alcohol self-administration in Wistar rats trained in an operant self-reinforcement paradigm. To further validate whether the loss of mGluR2 causally contributes to excessive alcohol intake, we examined alcohol-drinking behavior in *Grm2* knockout mice. Tested by a two-bottle free choice scheme and an escalation procedure, *Grm2*-null mice showed significantly higher levels of alcohol consumption and preference than the wild-type control.

To examine gene expression changes related to alcohol-drinking behavior, we also performed hippocampal transcriptome analysis in P and NP rats (Fig. 10.2C) using RNA-sequencing (Zhou et al., 2013). The results indicated an overall pattern of altered expression of genes involved in neural development and synaptic functions. A total of 485 genes were differentially expressed at FDR<0.05 following correction for genome-wide testing. Differentially expressed genes were significantly enriched with segregating SNPs located in the coding regions and UTRs, indicating the potential involvement of *cis*-regulatory elements in these genes. Using functional annotation analysis with twofold enrichment as a cutoff, we identified several functional domains among the 485 differentially expressed genes, including calmodulin binding, synapse, and neuronal projection. Of particular interest was overrepresentation of the genes that function in glutamate, GABA, opioid, cholinergic, and adrenergic transmission (Fig. 10.2C). This pattern was consistent with the loss of mGluR2 receptor in P rats, but also more readily points to overall neuronal differences between P and NP rats that influence alcohol-drinking behaviors. The gene expression differences between P and NP rats are thus consistent and convergent with their genetic and phenotypic differences and are likely to be influenced by their overall genetic differences or the interaction of *Grm2* C407* with other loci.

6. EPIGENETIC REGULATION OF GENE EXPRESSION IN ADDICTED BRAIN

Epigenetics plays a key role in regulating gene expression. Studies have shown that drug exposure causes changes in DNA methylation that lead to alterations in transcription. Acute cocaine treatment was reported to increase the expression of DNA methyltransferase genes, *Dnmt3a* and *Dnmt 3b*, in mouse NAc, resulting in DNA hypermethylation and the increased binding of methyl CpG binding protein 2 (MeCP2) at the promoter of protein phosphatase-1 catalytic subunit gene (*Pp1c*). As a result, *Pp1c* expression was decreased (Anier, Malinovskaja, Aonurm-Helm, Zharkovsky, & Kalda, 2010). In contrast, chronic cocaine administration was found to decrease expression of *Dnmt3a*. The attenuation of DNA methylation led to potentiated cocaine reward (LaPlant et al., 2010). Acute and repeated cocaine administration was also shown to cause hypomethylation at the FosB promoter, leading to upregulation of FosB expression (Anier et al., 2010). It has also been shown that in heroin addicts, there was elevated methylation at several CpG sites in the promoter of a μ-opioid receptor gene, *OPRM1*, in lymphocytes, which might result in reduced expression of that gene (Nielsen et al., 2009). Differential DNA methylation at the promoter of the pro-opiomelanocortin gene (*POMC*) was found to be associated with alcoholism in a human study (Muschler et al., 2010). DNA hypomethylation was associated with activation of endogenous retroviruses in alcoholic brain (Ponomarev et al., 2012).

Chronic drug exposure also causes significant changes of histone modification. Histone acetylation is known to be associated with activated gene expression. Chronic cocaine exposure was shown to inhibit the function of *Hdac5*, a histone deacetylase, in mouse NAc (Renthal et al., 2007). Activation of dopamine D1 receptor induced upregulation of histone acetylation at the promoters of tyrosine hydroxylase (*Th*) and brain-derived neurotrophic factor (*Bdnf*) genes in mouse NAc and the expression of the two genes (Schroeder et al., 2008). There was a reported association in mice between histone H3 acetylation-activated transcription of addiction-related genes, such as CamkII-α and the motivation for cocaine

(L. Wang et al., 2010). In mice chronically administered amphetamine, the Δ-FosB-mediated responses were also found to involve recruiting *Hdac1* to its target gene promoters (Renthal et al., 2008). Inhibition of histone deacetylase reduced behavioral sensitization to morphine in mice (Jing et al., 2011). Alteration of histone methylation also plays important roles in neuronal adaptation of addicted brain. Repeated cocaine administration in mice was shown to repress the expression of lysine dimethyltransferase G9a, resulting in decrease of histone lysine 9 dimethylation (H3K9me2) in NAc (Maze et al., 2010).

Our chromatin immunoprecipitation and genomic sequencing (ChIP-Seq)-based analysis in the postmortem hippocampus of cocaine addicts and alcoholics revealed significant changes in histone H3 lysine 4 trimethylation (H3K4me3) (Zhou et al., 2011), a histone mark known to be associated with activation of gene expression. Similar to the changes observed in gene expression, there was a more widespread and greater impact in response to chronic cocaine exposure than to alcohol exposure. There were also concordant changes between H3K4me3 and gene expression at some loci. In cocaine addicts, these included components of the mitochondrial oxidative phosphorylation pathway or regulators of cellular energy metabolism such as *NDUFS2*, *NDUFA12L*, *UQCRB*, *INSR*, and *IGF1R*; genes involved in LTP and other neuronal functions such as calmodulin 2 (*CALM2*), Synaptophysin-like protein 2 (*SYPL2*), sodium/chloride-dependent neurotransmitter transporter (*SLC6A15*), and nociceptin (*PNOC*). In alcoholics, there were concordant changes between H3K4me3 and gene expression of Protocadherin alpha-7 (*PCDHA7*), Aquaporin-11 (*AQP11*), and potassium inwardly-rectifying channel, subfamily J, member 5 (*CIR*), all of which are involved in critical neuronal and cellular functions. Globally, among all 13,113 histone H3K4me3 peaks mapped to the promoters of hippocampal expressed genes, there was a trend of correlation between H3K4me3 and expression changes in cocaine addicts. However, this trend was not observed in alcoholics. Overall, there was no significant overlap between the genes with either significant H3K4me3 changes or expression changes in both cocaine addicts and alcoholics. This may reflect the fact that epigenetic regulation of gene expression through chromatin remodeling involves many different types of histone modifications at many different histone residues (Barski et al., 2007; Wang et al., 2008), and cocaine- and alcohol-induced expression changes are very likely the results of alterations of those many different histone modifications.

Gene expression changes caused by chronic drug exposure may also be mediated by regulatory RNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs). Regulatory RNAs modify gene expression through multiple means, such as altering mRNA stability, basal transcription machinery, translational efficiency, and chromosome modification. miRNA array analysis in human prefrontal cortex revealed upregulation of approximately 35 miRNAs in alcoholics relative to controls with predicted target genes implicated in apoptosis, cell cycle, cell adhesion, nervous system development, and cell–cell signaling (Lewohl et al., 2011). Exposure of zebrafish embryos to cocaine reduced the expression of miR-133b in the CNS, and this difference in miR signaling might in turn modulate expression of dopamine receptors, the dopamine transporter, and tyrosine hydroxylase (Barreto-Valer, Lopez-Bellido, Macho Sanchez-Simon, & Rodriguez, 2012). The let-7 miR family may also interact with the 3′-untranslated region of μ-opioid receptor

mRNA to regulate opioid tolerance (He & Wang, 2012). miRNAs are both synaptically enriched and depleted by drug exposure. Cocaine modulates levels of the miR-8 family which is enriched at postsynaptic densities and regulates expression of cell adhesion molecules (Eipper-Mains, Eipper, & Mains, 2012). Differential expression of multiple lncRNAs was identified in the NAc of cocaine-conditioned mice and those lncRNAs were reported to regulate their target loci through both *cis*- and *trans*-actions (Bu et al., 2012).

7. CONCLUSION

Profiling gene expression in the addicted brain has revealed both agent-specific and common drug-induced neural adaptations, providing valuable insights for the understanding of the relevant molecular and cellular mechanisms. The development of transcriptome-based sequencing analysis has equipped us with potent tools that can be combined with neuroscience tools and approaches including the isolation of particular regions, circuits, and cells involved in addiction, genetic models including artificially selected strains and humans varying in vulnerability and response, and interventional models including pharmacological challenges and gene-based manipulations of pathway function and response. These approaches will further enable us to deconstruct the transcription machinery and epigenetic regulation in the addicted brain.

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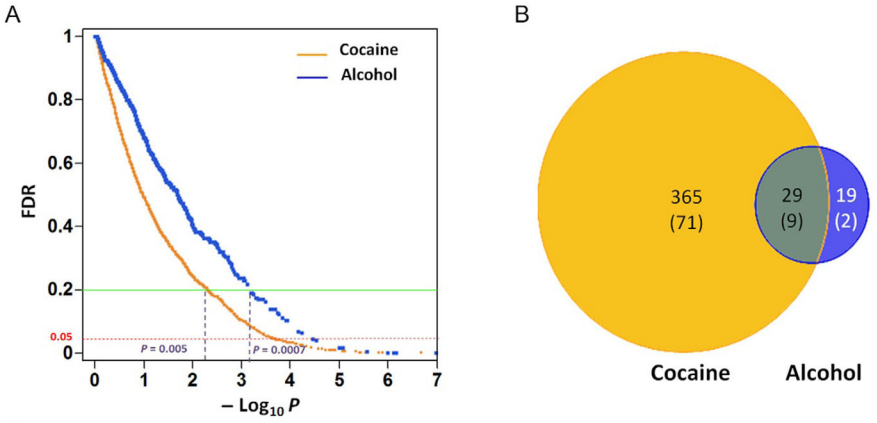


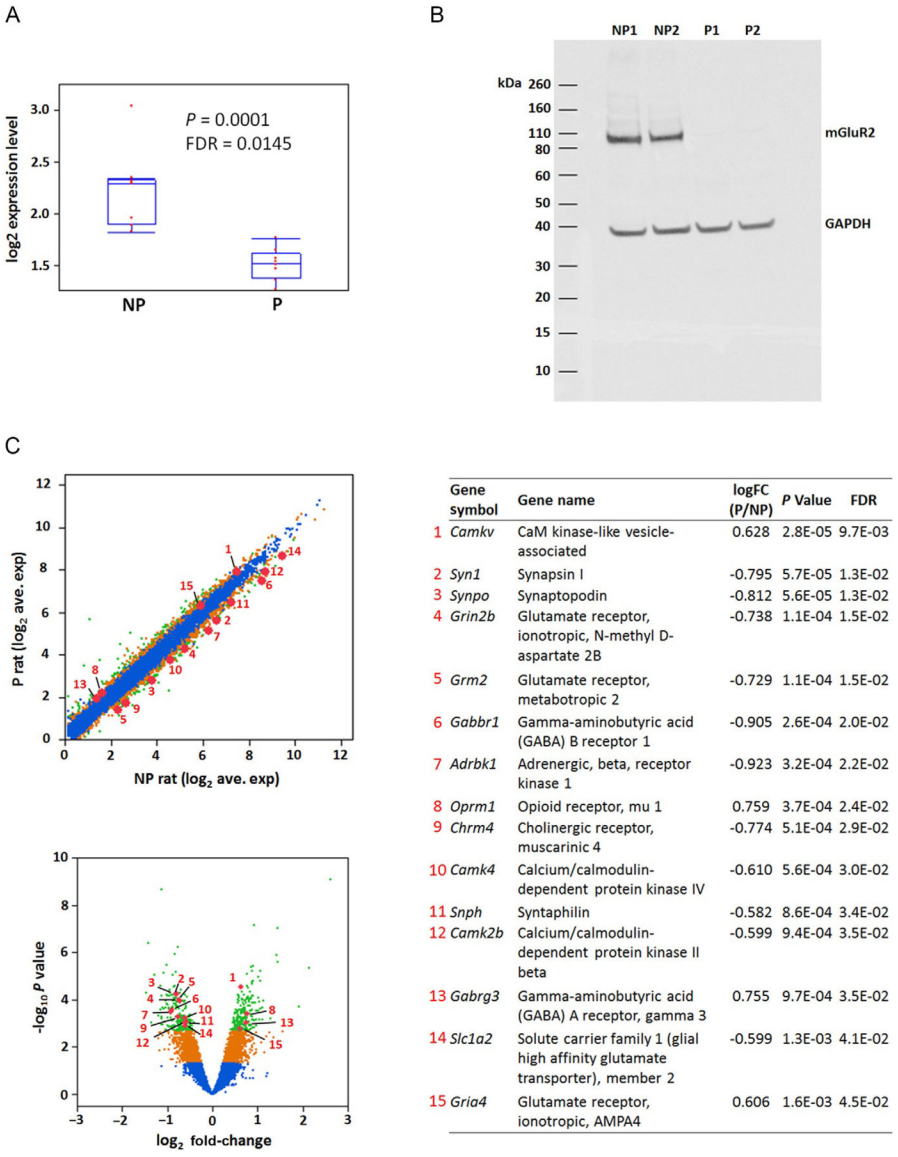
C

Genes with significant differential expression (FDR < 0.2) observed in both chronic cocaine- and alcohol-addicted individuals

Gene symbol	Gene name	Cocaine				Alcohol			
		log FC	*P* Value	FDR	Rank	log FC	*P* Value	FDR	Rank
HIST1H4E	Histone cluster 1, h4e	1.57	1.6E−10	0.000	*1*	1.70	2.5E−11	0.000	*1*
RN7SK	RNA, 7SK small nuclear	2.42	5.3E−10	0.000	*2*	1.31	3.6E−05	0.044	*11*
CDR1	Cerebellar degeneration-related protein 1, 34kda	1.81	5.2E−09	0.000	*3*	1.36	1.1E−06	0.004	*6*
SNORD89	Small nucleolar RNA, C/D box 89	1.53	5.6E−08	0.000	*5*	1.30	1.1E−06	0.004	*5*
SNORA73A	Small nucleolar RNA, H/ACA box 73A	1.41	2.2E−07	0.001	*6*	0.81	6.5E−04	0.190	*48*
SCARNA17	Small Cajal body-specific RNA 17	1.30	1.6E−06	0.004	*8*	1.08	3.0E−05	0.042	*10*
PAPD1	Mitochondrial poly(A) polymerase	0.95	4.1E−06	0.004	*14*	1.17	1.1E−07	0.001	*2*
CACNB2	Calcium channel, voltage-dependent, beta 2 subunit	0.87	4.8E−06	0.005	*15*	0.61	4.8E−04	0.171	*38*
LRCH4	Leucine-rich repeats and calponin homology (CH) domain containing 4	0.80	8.8E−06	0.008	*18*	0.65	1.5E−04	0.112	*16*
SNORD42A	Small nucleolar RNA, C/D box 42A	−1.47	1.2E−05	0.010	*19*	−1.48	1.1E−05	0.018	*9*
SNORA47	Small nucleolar RNA, H/ACA box 47	1.82	1.5E−05	0.010	*22*	1.48	2.3E−04	0.140	*21*
LENG8	Leukocyte receptor cluster (LRC) member 8	0.77	2.3E−05	0.014	*26*	0.72	6.2E−05	0.066	*12*
FAM123A	Family with sequence similarity 123A	0.72	3.9E−05	0.019	*30*	0.92	8.0E−07	0.004	*4*
HIVEP3	Human immunodeficiency virus type I enhancer binding protein 3	0.70	3.9E−05	0.019	*31*	0.59	3.3E−04	0.163	*26*
HNRPH1	Heterogeneous nuclear ribonucleoprotein H1	0.81	6.5E 05	0.026	*38*	0.72	2.7E−04	0.141	*25*
ZGPAT	Zinc finger, CCCH-type with G patch domain	0.55	9.2E 05	0.033	*43*	0.51	2.7E−04	0.141	*24*
ERF	Ets2 repressor factor	0.65	2.2E 04	0.046	*73*	0.82	8.7E−06	0.018	*8*
SNORD116-29	Small nucleolar RNA, C/D box 116-29	−1.68	3.0E 04	0.053	*89*	−1.82	1.2E−04	0.103	*14*
C9orf139	Chromosome 9 open reading frame 139	0.90	4.4E 04	0.068	*103*	0.89	5.2E−04	0.176	*41*
C9orf3	Aminopeptidase O	0.70	6.5E 04	0.084	*119*	0.73	4.0E−04	0.171	*29*
KCNA2	Potassium voltage-gated channel, shaker-related subfamily, member 2	0.67	1.1E 03	0.106	*153*	0.74	3.7E−04	0.171	*27*
EXOC6B	Exocyst complex component 6B	0.51	1.4E−03	0.124	*180*	0.55	6.4E−04	0.190	*45*
CENTB5	Arfgap with coiled-coil, ankyrin repeat and PH domains 3	0.54	1.8E−03	0.139	*198*	0.61	4.7E−04	0.171	*37*
TAOK2	TAO kinase 2	0.56	1.8E−03	0.139	*201*	0.68	2.4E−04	0.141	*22*
TNRC6C	Trinucleotide repeat containing 6C	0.59	2.8E−03	0.170	*255*	0.69	6.5E−04	0.190	*47*
ADAMTS4	ADAM metallopeptidase with thrombospondin type 1 motif, 4	0.65	2.9E−03	0.173	*263*	0.79	4.3E−04	0.171	*33*
MSH4	Muts homolog 4 (E. Coli)	0.79	3.5E−03	0.180	*302*	0.99	4.2E−04	0.171	*31*
C16orf72	Chromosome 16 open reading frame 72	0.54	4.3E−03	0.189	*358*	0.66	6.0E−04	0.186	*44*
CCR5	Chemokine (C-C motif) receptor 5	−0.79	4.7E−03	0.194	*386*	−1.19	7.4E−05	0.070	*13*

Figure 10.1.

Differentially expressed genes in the hippocampus of cocaine- and alcohol-addicted individuals, as detected by RNA-Seq. (A) Scatter plot of $-\log_{10} P$ (uncorrected *P* value) versus FDR *q* value for all 16,008 expressed genes. FDR thresholds of 0.2 and 0.05 are marked, as well as corresponding uncorrected *P* values at an FDR of 0.2. (B) Genes differentially expressed (FDR <0.2) in cocaine only, alcohol only, and common to both. (C) Genes with significant differential expression (FDR <0.2) observed in both chronic cocaine- and alcohol-addicted individuals.



	Gene symbol	Gene name	logFC (P/NP)	*P* Value	FDR
1	*Camkv*	CaM kinase-like vesicle-associated	0.628	2.8E-05	9.7E-03
2	*Syn1*	Synapsin I	-0.795	5.7E-05	1.3E-02
3	*Synpo*	Synaptopodin	-0.812	5.6E-05	1.3E-02
4	*Grin2b*	Glutamate receptor, ionotropic, N-methyl D-aspartate 2B	-0.738	1.1E-04	1.5E-02
5	*Grm2*	Glutamate receptor, metabotropic 2	-0.729	1.1E-04	1.5E-02
6	*Gabbr1*	Gamma-aminobutyric acid (GABA) B receptor 1	-0.905	2.6E-04	2.0E-02
7	*Adrbk1*	Adrenergic, beta, receptor kinase 1	-0.923	3.2E-04	2.2E-02
8	*Oprm1*	Opioid receptor, mu 1	0.759	3.7E-04	2.4E-02
9	*Chrm4*	Cholinergic receptor, muscarinic 4	-0.774	5.1E-04	2.9E-02
10	*Camk4*	Calcium/calmodulin-dependent protein kinase IV	-0.610	5.6E-04	3.0E-02
11	*Snph*	Syntaphilin	-0.582	8.6E-04	3.4E-02
12	*Camk2b*	Calcium/calmodulin-dependent protein kinase II beta	-0.599	9.4E-04	3.5E-02
13	*Gabrg3*	Gamma-aminobutyric acid (GABA) A receptor, gamma 3	0.755	9.7E-04	3.5E-02
14	*Slc1a2*	Solute carrier family 1 (glial high affinity glutamate transporter), member 2	-0.599	1.3E-03	4.1E-02
15	*Gria4*	Glutamate receptor, ionotropic, AMPA4	0.606	1.6E-03	4.5E-02

Figure 10.2.

Differential gene expression in hippocampus of P and NP rats. (A) Hippocampal *Grm2* mRNA levels in P and NP rats. (B) Western blot of hippocampal mGluR2 protein in NP and P rats (2 individuals in each group). (C) Average expression levels (normalized $\log_2$ read counts, upper left) of 11,406 genes measured by RNA-Seq are compared between P and NP rats. The fold change (P vs. NP) and nominal *P* values ($-\log_{10}$, *t* test) for each gene are also plotted (lower left). Significance of difference between P and NP for each gene is color coded: blue (black in the print version), not significant; orange (dark gray in the print version), $P<0.05$; green (light gray in the print version), false discovery rate <0.05. Fifteen differentially expressed genes among those in the over represented functional domains are highlighted and details of their expression differences are listed in the table (right).