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MicroRNAs in addiction: adaptation's middlemen?

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Abstract

A central question in addiction is how drug-induced changes in synaptic signaling are converted into long-term neuroadaptations. Emerging evidence reveals that microRNAs (miRNAs) have a distinct role in this process through rapid response to cellular signals and dynamic regulation of local mRNA transcripts. Because each miRNA can target hundreds of mRNAs, relative changes in the expression of miRNAs can greatly impact cellular responsiveness, synaptic plasticity and transcriptional events. These diverse consequences of miRNA action occur through coordination with genes implicated in addictions, the most compelling of these being the neurotrophin *BDNF*, the transcription factor cAMP-responsive element-binding protein (*CREB*) and the DNA-binding methyl CpG binding protein 2 (*MeCP2*). In this study, we review the recent progress in the understanding of miRNAs in general mechanisms of plasticity and neuroadaptation and then focus on specific examples of miRNA regulation in the context of addiction. We conclude that miRNA-mediated gene regulation is a conserved means of converting environmental signals into neuronal response, which holds significant implications for addiction and other psychiatric illnesses.

Keywords

addictions; mechanisms; miRNA; plasticity; regulation

Introduction

The past decade has seen a significant shift in the conceptualization of the genome. Non-coding elements, once considered 'junk sequences' (that is, evolutionary relics), are now known to be important regulators of gene expression necessary for the development and organization of complex life.[1] This new framework allows extensive novel investigation into non-coding RNA, as 98% of the human genome is non-protein coding.[2] One such class of non-coding RNAs, called microRNAs (miRNAs), are short (22 nucleotides), evolutionarily conserved regulatory molecules that directly target the 3′-untranslated region (3′-UTR) of mRNAs to modulate gene expression post-transcriptionally.[3] miRNAs are endogenous to mammalian cells[4] and are essential controllers of cellular proliferation, differentiation and

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Conflict of interest
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apoptosis.[5,6] In humans, more than 1000 miRNA sequences have thus far been identified.[7] More than a third of all genes are subject to miRNA regulation, with each miRNA family targeting an average of ~500 RNA transcripts.[8]

Several properties of miRNA regulation and processing make them ideally suited for rapid environmental response. First, their small size and non-coding nature allow them to be transcribed more quickly than other immediate-early response genes, which are much longer and must undergo the additional step of translation.[9] Second, because miRNAs target mRNAs directly, they regulate protein synthesis at the ribosome. Furthermore, their association with the ribosome allows subcellular localization, including to dendrites.[9,10] The localization of miRNAs and their processing machinery to dendritic compartments provide a means for altered gene regulation in direct response to synaptic activity, fulfilling a unique requirement of neurons for synapse-specific adaptation as distinct from cell-wide changes in gene expression.[11]

Given their ubiquitous nature as well as the enrichment of many miRNAs in the brain,[12,13] it is not surprising that they have been implicated in an ever-increasing number of neurological diseases. During the past several years, miRNA involvement has been associated with central nervous system disorders such as Tourette's syndrome[14] and Rett syndrome;[15] neurodegenerative diseases such as Parkinson's,[16] Huntington's,[17] and Alzheimer's disease[18,19] and psychiatric disorders such as schizophrenia[20,21] and addiction.[22,23] An important role for miRNAs in addiction is supported by their established role in synaptic plasticity. Long-term facilitation, wherein neuronal synapses alter in strength according to activity at the synapse, is regarded as the underlying mechanism of addiction, compulsion and dependence,[24-26] and drugs of abuse alter synaptic signaling in various brain regions, particularly the ventral tegmental area,[27] striatum,[28] nucleus accumbens[29] and prefrontal cortex.[30] miRNAs, by their modes of expression and action, are thus uniquely equipped to respond to altered synaptic signaling and induce neuroadaptation.

miRNAs in synaptic plasticity and neuronal regulation

The extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) family is a class of signal-transducing enzymes activated by cell-surface receptors and chemical or physical stresses.[31] MAPK/ERK signaling regulates local miRNA expression via phosphorylation of the miRNA-generating complex,[32] providing a common means through which an extracellular signal can be rapidly converted to an miRNA-mediated response. There are two general types of adaptive response under miRNA regulatory control: direct regulation of protein synthesis by miRNAs has a crucial role in plasticity at the synapse,[33,34] whereas interactions of miRNAs with transcription factors seem to modulate more enduring neuroplastic changes on the level of the entire cell.

Dendritic morphology

Dendritic miRNAs may underlie or enhance the observed effects of key molecules in synaptic plasticity. Brain-derived neurotrophic factor (BDNF) is a neurotrophin crucial to cortical survival and maintenance, as well as the growth of new neurons and synapses.[35,36] BDNF can induce transcription of miRNA-containing gene loci[37] and interact directly with

mature miRNAs.[38] Treatment of neonatal rat cortical cells with BDNF upregulates the miRNA precursor premiR-132 through its action on cAMP-response element binding protein (CREB).[39] In turn, mature miR-132 stimulates neurite outgrowth (the process preceding synapse formation) through inhibition of p250GAP, a protein that represses neurogenesis.[39] Increased thickness of dendritic spines from transgenic miR-132 has been confirmed by an *in vivo* study.[40] Another miRNA-mediated role for BDNF has been found in post-natal rat hippocampal cells: the brain-specific miR-134, located in the synaptodendritic compartment, inhibits translation of co-localized Lim domain-containing protein kinase 1.[38] Lim domain-containing protein kinase 1 is a regulator of actin filament dynamics, necessary for dendritic spine development.[41] Treatment with BDNF releases miR-134's inhibition of Lim domain-containing protein kinase 1, thereby stimulating growth of the dendritic spine. This mechanism is reversible, with the activity state of the synapse switching translational inhibition on or off,[38] illustrating a dynamic role for this miRNA in synapse plasticity.

Two recent studies[42,43] provide additional evidence of localized miRNAs affecting dendritic structure. In the first, miR-138 was shown to restrict dendritic growth in rat hippocampal neurons through inhibition of acyl protein thioesterase 1.[42] Calcium influx decreased pre-miR-138 levels and miR-138 cleavage activity, facilitating dendritic strengthening in response to synaptic stimulation. In a study of fragile-X mental retardation protein-related miRNAs in mouse hippocampal neurons, overexpression of either miR-132 or miR-125b led to opposing dendrite morphologies, with miR-132 corresponding to thicker spines and miR-125b to thinner.[43] Interestingly, knockout of fragile-X mental retardation protein prevented these effects despite the lack of an miRNA recognition site, indicating an indirect association that regulates downstream protein targets in tandem.[43] Taken together, these findings provide evidence that localization of miRNAs to synapses and their subsequent regulation of protein synthesis in response to specific synaptic stimuli is a significant mechanism underlying plasticity.

Gene regulation and memory

miRNA targeting of transcription factors such as CREB provides an additional layer of regulation with more widespread and enduring neuronal consequences. CREB-induced transcription is an important component of a switch from short-term to long-term plasticity,[44] and proper CREB functioning is necessary for long-term memory formation.[45] Heightened CREB concentrations increase neuronal excitability in the amygdala and nucleus accumbens,[46,47] and the degree of CREB phosphorylation has been associated with sensitization to cocaine[48] and morphine.[49]

Comparative sequence analysis reveals that miR-NAs expressed in neurons are highly enriched with cAMP-response elements and neuron-restrictive-silencing elements, implicating CREB as a positive regulator and RE1-silencing transcription factor (REST) as a common repressor of these genes.[50] Because some neuronal miRNAs also target CREB and REST, and because all three types of regulators share neural gene targets, it is proposed that a network among CREB, REST and miRNAs carries out coordinated gene regulation through extensive feedback. Modeled gene networks indicate that feedback circuits increase

the stability and robustness of the system,[51] and mutual binding sites and targets shared by miRNAs and transcription factors could be seen as enabling cross-talk between the levels of genome, transcriptome and proteome.

The most abundant miRNA in the brain, miR-124,[52] shows mutual targeting with REST. This antagonistic relation is important to cellular differentiation and identity through opposing effects on neural and non-neural transcripts,[53] with miR-124 promoting a neuronal phenotype.[54] In mature neurons, miR-124 also inhibits CREB in an activity-dependent manner.[55] miR-124 responds to the neurotransmitter serotonin (5-HT) in Aplysia neurons with a rapid decrease in expression, leading to an increase in CREB expression and inducing long-term facilitation.[55] Under normal conditions, this effect required five spaced pulses of 5-HT; however, when miR-124 was downregulated or CREB was upregulated, long-term facilitation could be induced after a single pulse. Thus, miR-124 and CREB can be seen to work in conjunction to mediate neural responsiveness to serotonin-induced learning.

Gao and colleague[56] identified another miRNA–CREB pathway important in memory. Deficiency of SIRT1 was found to impair plasticity and memory formation in mice and cause overexpression of miR-134, an miRNA previously implicated in dendritic morphology. miR-134 was predicted to have three binding sites in the 3′-UTR of CREB mRNA, and luciferase reporter assays confirmed direct binding. It was found that SIRT1 normally forms an inhibitory complex upstream of the *miR-134* gene to regulate its expression negatively. When disinhibited, overexpression of miR-134 downregulates CREB and BDNF (which is CREB activated) and leads to impaired plasticity and memory deficits. Blocking miR-134 in SIRT1-knockout mice was able to reverse these deficits.[56] Intriguingly, Renthal *et al.*[57] reported that chronic, but not acute, cocaine exposure increases the expression of SIRT1 in the nucleus accumbens, and that SIRT1 inhibition decreases the rewarding effects of the drug. Cocaine's upregulation of SIRT1 could conceivably exert the observed effects via the miR-NA–CREB pathway discussed.

Chromatin remodeling may represent an additional component of a coordinated mechanism with miRNAs and transcription factors through which signaling-induced neuroadaptations gain long-term stability. miR-132, the CREB-activated miRNA involved in dendrite morphogenesis, also orchestrates chromatin remodeling through regulation of MeCP2, p300 and JARID1A in the suprachiasmatic nucleus, with the effect of attenuated resetting of the circadian clock in response to light.[58] MeCP2 is a DNA-binding protein that can compact chromatin structure,[59] repress transcription by competitive binding at promoters or through complex formation with histone deacetylases or co-repressors,[60] or activate transcription through association with CREB1.[61] MeCP2 is abundantly expressed in neurons and is critical to proper functioning; its over- and under-expression both result in detrimental neural effects, and mutations in the MeCP2 underlie Rett syndrome.[62] There is recent evidence that MeCP2 regulates a cohort of miRNAs (including miR-132) through binding at promoter regions of miRNA transcription units, where it acts primarily as a repressor.[63] Several of these miRNAs were found to be synaptically enriched, and many were also predicted to target BDNF, which is downregulated in MeCP2-knockout mouse and rescues Rett syndrome-like deficits.[64] In turn, miR-132 represses MeCP2 but is activated by BDNF,

highlighting an miRNA autoregulatory loop,[63] which apparently stabilizes activity-dependent BDNF production, as well as MeCP2 expression.[65]

miR-132, CREB, MeCP2 and BDNF have all been demonstrated to be important components of learning and memory.[40,45,66,67] An emerging picture is that these distinct molecular entities form a multi-level network that responds to neural activity at the immediate level of protein functioning at the synapse, but also, uses continuing feedback at the transcriptional and post-transcriptional level to carry out longer-term changes necessary for memory formation. The precise relations of such complex epigenetic networks are yet to be elucidated—particularly at the level of the miRNA, where the vast number of potential targets presents a practical challenge. However, miRNAs seem to occupy a unique position between synaptic signaling and neuronal gene expression, which holds significant consequences for memory as well as addiction.

miRNAs in substance use disorders

As drug addictions are widely regarded as disorders of plasticity, according to reward-based learning,[24] it could be expected that miRNA-mediated mechanisms of synaptic plasticity such as those just described in functional systems contribute to formation of the addictive phenotype. During the past few years, evidence has begun to accumulate that miRNA responses to drug-induced stimuli have important roles in neuroadaptive pathways that are induced by, or react against, consistent drug exposure.

Cocaine addiction

A recent study by Hollander and colleagues[68] reported altered miRNA expression in the striatum, a brain region involved in drug-seeking habits.[69] Increased expression of miR-132 and the closely related miR-212 were observed in rats having extended access (6h per day), but not in rats under restricted access or ‘yoked’ rats, who received cocaine in a response-independent manner. Increasing or decreasing miR-212 expression was found to decrease and increase cocaine self-administration under unlimited access, indicating that this miRNA decreases cocaine’s motivational properties and protects against over-consumption. miR-212 appears to exert this effect, at least in part, by upregulating striatal CREB.[68] Upregulation of the cAMP pathway is a compensatory response to chronic drug exposure,[70] and elevation of CREB in the nucleus accumbens decreases the rewarding effects of cocaine.[71] Follow-up work from the same research group[72] demonstrated that MeCP2 forms a homeostatic interaction with miR-212 to control BDNF expression and cocaine intake. MeCP2 attenuates cocaine’s upregulation of miR-212 and subsequent CREB signaling, whereas miR-212 inhibits MeCP2 expression. Although there is evidence that MeCP2 itself acts as a transcriptional repressor of BDNF in the absence of neuronal activity,[62] MeCP2 levels coordinate closely with BDNF levels in the brain,[73] and phosphorylation of MeCP2 regulates activity-dependent expression of BDNF.[74] Together, these observations indicate that a BDNF-MeCP2-inclusive network such as that described previously is necessarily co-expressed in response to neural activity and is engaged by cocaine. BDNF expression in the nucleus accumbens produces robust behavioral consequences, facilitating compulsive cocaine-taking behavior and increasing measured cocaine reward.[7576] As CREB induces

both BDNF[77] and MeCP2[65] expression, it seems that miR-212, by suppressing MeCP2 (and subsequently BDNF), serves as a 'filter' for CREB-responsive genes.[72]

The upregulation of miR-212 in the striatum may reflect a mechanism of tolerance within a neuron. Each use of cocaine upregulates BDNF,[78] and BDNF action on its TrkB receptor is one of several types of synaptic activity that induces transcription of miR-212 and miR-132,[37] which accounts for the observed increase in miR-212 in rats having extended access to cocaine. A sustained increase would reduce normative activity-dependent BDNF expression, which would decrease the rewarding effects of each cocaine exposure. More cocaine would therefore be necessary to achieve the same effect (Figure 1).

Chandrasekar and Dreyer[79] applied miRNA prediction software to identify miRNAs that might target cocaine-responsive genes implicated in addiction and found strong prediction for miR-124, let-7d and miR-181a. miRNA quantification of rat mesolimbic brain slices showed that miR-124 and let-7d were significantly downregulated and miR-181a was significantly upregulated by chronic cocaine administration. Further investigation[80] revealed that overexpression of miR-124 and let-7d in the nucleus accumbens attenuates cocaine-induced conditioned place preference, whereas miR-181a overexpression enhanced cocaine-induced conditioned place preference, and silencing of these miRNAs produced inverse effects. This study further demonstrated an impressive array of addiction-related gene expression changes in these various conditions. Notably, miR-124 and let-7d overexpression upregulated the dopamine transporter, whereas miR-181a overexpression downregulated it. Because dopamine transporter is cocaine's directly inhibited target and the source of its effects on the dopaminergic system,[81] these findings likely relate strongly to the observed effects of manipulation of these miRNAs on conditioned place preference, an indirect measure of cocaine reward, and reflect compensatory changes in the cases of miR-124 and let-7d, and a sensitizing change in the case of miR-181a. The expression of a number of other genes is modulated by these miRNAs, including ΔFos and Fos B, DRD2 and DRD3, Nac1, Per2, GRIA2 and 7MYT1, highlighting the diverse effects of miRNA dysregulation on synaptic signaling (via receptors) and transcription factors. In light of the networks examined in this report, the effects of these miRNA manipulations on BDNF, CREB and MeCP2 are of particular interest. BDNF expression was decreased when miR-124 was silenced, or when let-7d was either overexpressed or silenced; MeCP2 was significantly downregulated when any of the three miRNAs was silenced, with the strongest effect (a 10-fold decrease) in the case of miR-124. Amounts of CREB protein also increased significantly when miR-124 was silenced.[81]

Although the transcriptional repressor REST was not examined in the more recent investigation, the initial study by Chandrasekar and Dreyer[79] found that chronic cocaine exposure induces REST expression. The previously discussed antagonistic relation between miR-124 and REST might reasonably imply that this is another instance of a homeostatic relation between an miRNA and a transcription factor as an adaptation to chronic cocaine exposure. miR-124 and REST both target and suppress BDNF, so it seems that REST induction might serve to transfer control of BDNF inhibition from the translational level (via miRNA regulation) to the transcriptional level (via inhibition by REST). This shift may be necessary to allow an adaptive increase in CREB that was observed in the miR-124

silencing condition, as CREB is targeted by miR-124 but not REST (Figure 2). An inverse transfer of BDNF regulatory control has been observed in the prefrontal cortex: BDNF mRNA has been found to correlate more strongly with mechanisms of transcriptional control (for example, open-chromatin-associated histone H3 methylation) during childhood, whereas miRNAs became more prominent regulators in adolescence and adulthood.[82] As miR-124 promotes the neuronal phenotype whereas REST opposes it, the observed effects of cocaine on these factors might be postulated to represent a regression into a more 'immature' neuronal phenotype. Observed effects of chromatin remodeling of the *BDNF* gene in response to chronic but not acute cocaine treatment[83,84] also support such a shift in control. Regardless, the combined effects on CREB are likely relevant to learning-related aspects of addiction, such as cocaine-induced cues,[80] as well as general cocaine reward.[71]

Whereas greater amounts of CREB protein in the nucleus accumbens decrease cocaine self-administration and relapse,[85] more BDNF in this region increases self-administration and relapse.[86] This is a somewhat surprising disparity, given that CREB induces BDNF transcription, but appears to be indicative of a general counter-balance between adaptations that accentuate cocaine's signaling effects and those that offset these effects (even within the same network). miRNAs such as miR-124 and miR-212 appear to mediate this balance by selective targeting and activity-dependent expression.

Nicotine dependence

A characteristic shared feature of addictive drugs is an unconditional increase in synaptic dopamine,[87] so dopamine receptor expression represents a potentially important factor in drug response. We recently investigated the differential expression of the dopamine receptor *D1* gene (DRD1) in response to nicotine.[23] This gene was previously found in a genetics association study to contain a single-nucleotide polymorphism (rs686) significantly associated with nicotine dependence (ND).[88] Because the polymorphism rs686 was in the 3′-UTR, we hypothesized that such a significant genetic association of the polymorphism with ND might be mediated by miRNA. Investigation of candidate miRNAs revealed that mir-504 directly targeted *DRD1*, with the surprising effect of upregulating expression. Moreover, upregulation was significantly greater with the 'A' allele associated with ND. This observed effect agrees with the stronger predicted binding energy of miR-504 to the transcript containing this allele, as was confirmed by assay with an miR-504 inhibitor.[23] The role of dopamine signaling in reward and motivation suggests that this miRNA-mediated pathway may underlie continued smoking behavior by increasing dopamine D1 receptor synthesis at nicotine-affected synapses. It may also affect plasticity downstream, as D1-receptor signaling phosphorylates CREB,[89] and phosphorylated CREB in the nucleus accumbens is necessary for nicotine-induced conditioned place preference.[90]

In another study, we used an miRNA microarray approach to investigate the broad effects of nicotine stimulation on miRNA expression in rat PC12 cells.[22] From several hundred probe sets, 25 miRNAs were found to show significant changes, evidence that nicotine exerts specific but widespread effects on miRNA regulation. One of these, miR-140*, showed a strong predicted binding site on dynamin1 (Dnm1), a large GTPase important for synaptic endocytosis that is significantly associated with ND.[91] We subsequently demonstrated that

this miRNA is greatly upregulated in response to nicotine treatment and binds directly to Dnm1 to inhibit its expression.[22] Dynamin 1 may have a key role in chemical dependence through its action in signal termination of G-protein-coupled receptors, which include dopamine and opioid receptors, as changes in sensitivity of these receptors underlie acute drug effects.[92] Moreover, morphine has been distinguished from non-addictive analogs by exhibiting a deficient ability to induce endocytosis of its receptor, which disrupts signal termination and desensitization.[93] As dynamin 1 is crucial to endocytosis of G-protein-coupled receptors,[94] its downregulation by miR-140* might contribute to the highly addictive aspects of nicotine, such as tolerance and craving.

Interestingly, several of the miRNAs found in this study to undergo nicotine-induced changes in expression also have been implicated in schizophrenia and other neurodegenerative disorders. For example, miR-181b, which is upregulated by nicotine, is upregulated in the temporal cortex of schizophrenic patients,[95] whereas miR-30a-5p (one of the miRNAs that target BDNF in the prefrontal cortex[82]) and miR-29c are both downregulated by nicotine and are downregulated in the prefrontal cortex of postmortem brains in schizophrenia.[20] As schizophrenia and ND show a strikingly high degree of co-morbidity,[96,97] a more directed study of shared miRNA mechanisms in the two disorders could be very telling; it is possible that miRNAs could account for the co-morbidity, either through exacerbation of psychotic symptoms in response to drug use or by exerting similar effects in response to both anti-psychotics and cigarettes, thus fitting with a self-medication hypothesis.

miRNAs also appear to link nicotine with Alzheimer's disease. miR-125b was found to be upregulated by nicotine and in the hippocampus of Alzheimer's disease (AD) patients,[98] whereas miR-93, upregulated by nicotine, is downregulated in the cortex in AD.[99] Perhaps the most compelling correlate is miR-328, which is upregulated by nicotine and appears to have a significant role in the etiology of AD. Studies using postmortem tissues have revealed higher concentrations of β-amyloid precursor protein-converting enzyme protein (BACE1) in the brains of AD patients,[100,101] which leads to the build-up of β-amyloid, a major component of senile plaques etiologic of AD, thought to be responsible for neurodegeneration.[102] BACE1 is a predicted target of miR-328, and in a rodent model of AD, this miRNA was found to target and suppress BACE1 expression.[103] Because nicotinic receptor stimulation protects neurons against β-amyloid toxicity,[102] it is tempting to speculate that miR-328 upregulation by nicotine may be a component of the pathway underlying this protective effect. Regardless, the appearance of nicotine-responsive miRNAs in the etiology of neuropsychiatric disorders generally supports a role for these regulators in neural functioning. Table 1 provides a list of miRNAs dysregulated both in response to drugs and in neuropsychiatric illness.

Alcoholism

Alcohol exposure induces differential expression of about 2% of miRNAs in murine liver.[104] Many of these miRNAs are also expressed in the brain, so it will be important to see whether alcohol exerts similar effects in this context.[33] In murine striatal neurons and in adult rat neurons, miR-9 undergoes significant upregulation in response to alcohol and

appears to contribute to alcohol tolerance through its regulation of the BK channel.[105] This channel is highly relevant to neuronal function, as it regulates excitability, shaping of action potentials and neurotransmitter release.[33,106] In mammals, alcohol evokes tolerance of BK channels.[107] Intriguingly, miR-9 preferentially targets and degrades transcripts of BK channel isoforms sensitive to alcohol potentiation, whereas transcripts encoding alcohol-tolerant channels tend to lack miR-9-binding sites in their 3′-UTRs.[33] Thus, alcohol-induced upregulation of miR-9 shifts BK channel expression toward more tolerant isoforms. miR-9 also targets DRD2,[33] and lower expression of this receptor has been associated with alcohol abuse,[108] indicating that this miR-9 might influence the rewarding effect of alcohol in addition to its involvement in tolerance.

A systems genetic analysis of alcohol consumption has found that variations underlying GABAergic brain function contribute a significant genetic component, and that G protein subunit beta 1 (Gnβ1) represents a candidate transcript for miRNA regulation relevant to alcohol consumption, based on differential 3′-UTR sequences (and predicted binding affinities) between various intensities of alcohol consumption.[109] The strongest target predictions across multiple software platforms were for miR-101a/b and miR-218. Subsequent studies to investigate the actual effects of these miRNAs on alcohol consumption will be necessary for confirmation. In support of a role for regulation of the GABAergic system in alcoholism, infusion of a $GABA_A$ alpha siRNA vector (pHSVsiLA2) into the central nucleus caused a reduction of binge drinking in alcohol-preferring rats.[110] This study represents a promising implementation of gene therapy given the successful behavioral effect and the tight control of the micro-infusion to specific brain regions.[110]

Perspective

From initial drug exposure to chemical dependence and addiction, there is a panoply of molecular changes that comprise neural adaptation. Recent studies on the effects of drugs of abuse on miRNAs reveal that these tiny regulatory molecules can have either a contributing role in the development of addiction, as in the case of miR-504 increasing *DRD1* expression, or a counteractive role against drug stimulatory effects, as in the case of miR-212 upregulating CREB. These converse biological effects represent the ‘pull’ and ‘push’ of addiction: the response of motivation-based learning networks toward perceived reward vs. the counter-response of neuronal homeostasis against sustained alterations in extracellular signaling. Although these effects of sensitization and tolerance are divergent, both appear to be multi-level adaptations, spanning from short-term changes in signaling cascades to long-term changes in baseline gene expression. miRNAs can respond to synaptic signals (for example, miR-124’s response to 5-HT) and regulate local protein synthesis, but also mediate transcription factors and chromatin remodelers; thus we propose that they are uniquely suited for neuroadaptation by converting short-term into long-term plasticity.

Plasticity relies on coordinated changes among vastly complex molecular networks, and drugs of abuse seem to exert their effect not via a single member of the network, but through coordination. However, one conserved mechanism among these gene networks appears to be miRNA-constrained feedback loops, wherein a drug-induced stimulus acts as an impetus for a change in gene expression through a temporary effect on an miRNA or transcription factor

before a balance is restored through feedback. Particularly among activity-dependent species, such as BDNF, CREB and MeCP2, in which precise spatiotemporal regulation is essential, feedback loops would be necessary for both stability and efficiency during complex, associative changes within the molecule. Furthermore, the observations of miRNA-CREB interactions in mediating neuronal responsiveness might suggest a role for miRNAs as markers of recent neural activity, thus providing a context for subsequent network activation. A more precise investigation of the temporally dependent effects of neuronal (and particularly hippocampal) miRNA expression compared with early activation genes might further elucidate this question.

A fuller characterization of miRNA species and their targets will be crucial to a fuller understanding of this type of gene regulation and to practical application. Deep sequencing studies are already allowing large-scale profiles of miRNA populations, but it also will be necessary to characterize differential miRNA expression among different cell types and at specific synapses to fully understand their functional roles. Given the growing specificity of our knowledge ofmiRNA targets, their ability to modulate numerous downstream targets makes them attractive as potential therapeutic targets, because such manipulations might affect an entire network rather than a single species. In addiction especially, the potential to inhibit the longer-term adaptations to drugs of abuse would be helpful in stopping the progression of the disorder and decreasing relapse risk. Of course, the enduring risks of such gene manipulations must be addressed more thoroughly in pre-clinical trials than we have yet seen.

Although miRNAs appear uniquely situated to participate in cross-talk between cellular signaling and long-term gene expression, the precise means and the degree of specificity of such a phenomenon is a mystery. Within a neuron, the relative level of expression of a gene, for example BDNF, will certainly affect its chromatin structure, in essence because the ’supply’ (transcription) of the gene must match the demand. Is it possible that the corresponding levels of the gene’s targeting miRNA, for example, miR-132, might also be factored into the gene’s chromatin remodeling? In this example, the answer seems to be yes, through miR-132’s regulation of MeCP2, which in turn regulates BDNF. The tantalizing possibility, however, is that something similar is happening on a much larger scale, perhaps using miRNAs combinatorially.

Although the full power of miRNAs as gene regulators remains to be seen, they certainly seem to have a significant role in proper brain functioning. Their adaptive nature is distinctly suited for a role in addiction, but their dysregulation is also being observed increasingly in schizophrenia, Parkinson’s and Alzheimer’s. As our ability to understand gene networks increases in both scope and precision, we will certainly want to be attentive to these tiny regulatory molecules, as the early evidence suggests they may serve as critical links.

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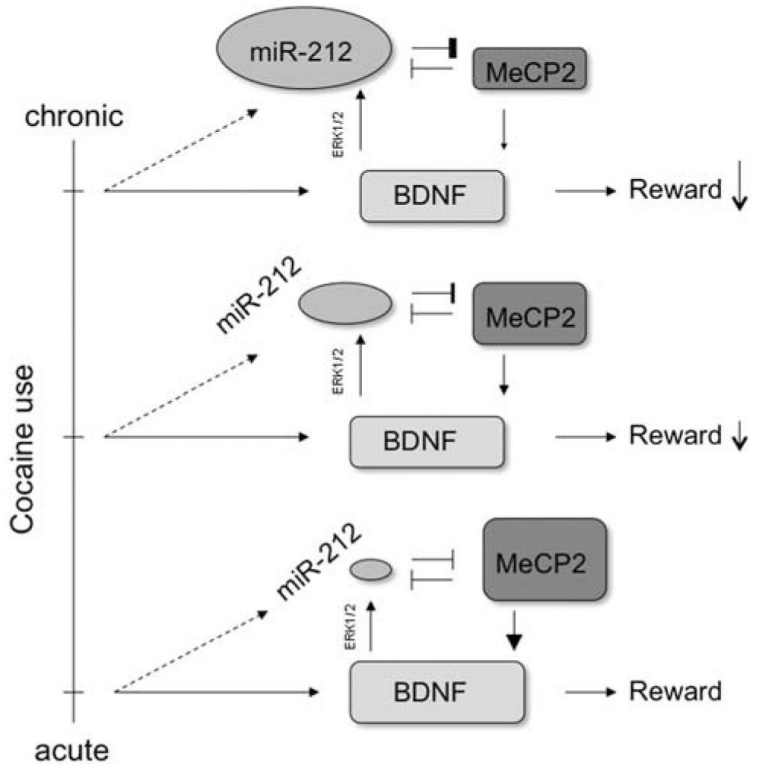

Figure 1.

Relation between miR-212, methyl CpG-binding protein (MeCP2), and brain-derived neurotrophic factor (BDNF) mediates the adaptive response to chronic cocaine exposure. Cocaine increases BDNF concentrations, even after a single dose,[78] and BDNF has a strong role in the motivating and rewarding aspects of the drug.[86] BDNF signaling at the synapse increases transcription of miR-212 via an extracellular-signal-related kinase (ERK1/2) pathway.[37] This miRNA exhibits mutual inhibition with MeCP2, a transcription factor necessary for BDNF expression in response to neural activity.[74] The increased expression of miR-212 observed after chronic cocaine treatment[72] therefore represents a mechanism of tolerance by inhibiting activity-dependent BDNF transcription in the nucleus accumbens.

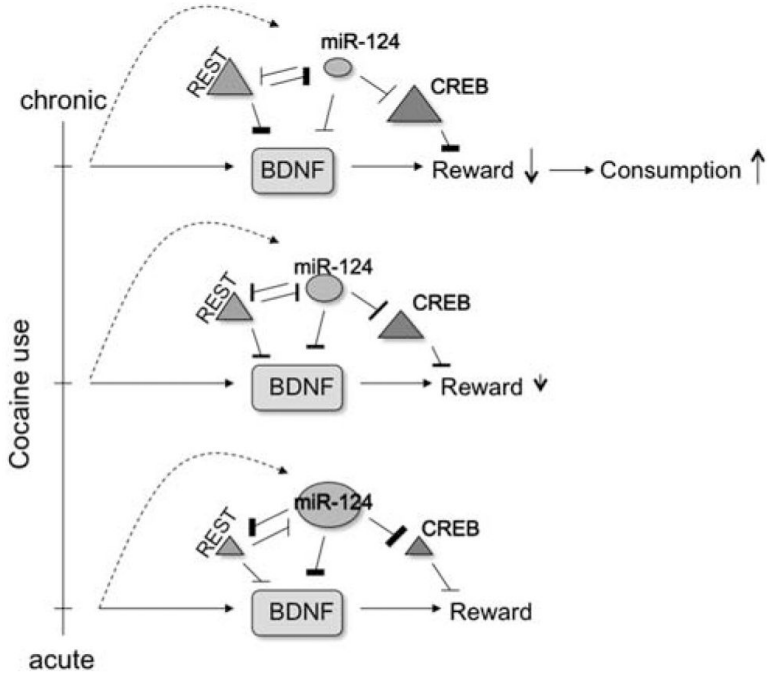

Figure 2.

Decreased miR-124 expression in chronic cocaine conditions allows sustained increases in cAMP response element-binding protein (CREB). miR-124 is important in learning and memory through its inhibition of CREB.[55] Following chronic cocaine exposure, this miRNA is down-regulated, whereas two of its targets, CREB and RE1-silencing transcription factor (REST), are upregulated.[79] Both miR-124 and REST inhibit BDNF expression, so it seems that downregulation of this miRNA marks a shift in the control of BDNF inhibition from miR-124 to REST. This shift allows higher concentrations of CREB, which decreases the rewarding effects of cocaine.[111]

Table 1

miRNAs responsive to drugs of abuse that have also been associated with neuropsychiatric disorders

miRNA	Targeted gene(s)	Biological function(s)	Associated diseases
mir-124	REST[53] CREB[55] BDNF[79]	Neuronal identity[53] 5-HT-induced learning[55] Plasticity[79]	Cocaine addiction,[79] Alzheimer's disease[17]
miR-132	P250GAP[39]	Neurogenesis[39]	Cocaine addiction,[68] Huntington's disease[17]
miR-181b	VSNL1[95] GLIA1[95]	Intracellular signaling[95] Neurotransmission[95]	Nicotine dependence,[22] schizophrenia[95]
miR-30a-5p miR-29c	BDNF[82]	Plasticity	
miR-125b	Lin-28[17] NR2A[43]	Neurogenesis[17] Neurotransmission/plasticity[43]	Nicotine dependence,[22] Alzheimer's disease[17]
miR-93 miR-328	VEGF[112] BACE1[103]	Cellular signaling[112] Axon guidance, potentiation[113]	ND,[22] Alzheimer's disease,[17] schizophrenia[113]

Abbreviations: miRNAs, microRNAs; ND, nicotine dependence.