

Epigenetic modifications and cellular mechanisms in the nucleus accumbens induced by chronic cocaine administration

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Abstract

Addiction is a large worldwide problem that occurs to many people and suffers their daily life. Most people will find that it can be very difficult to get out of the addictive state. The danger of developing an addiction to drugs, such as cocaine, is that tolerance is build. This leads to the fact that more and more of the substance is required to obtain the desired effect and eventually this desire is fully lost and replaced with a large craving for the drug, which is then taken simply to feel normal again. There are many therapies available nowadays, but it is still a large struggle towards succeeding. A recent interest is found in epigenetic modifications induced by drugs. It appears that chronic administration of a drug leads to modifications in the epigenetic pathways in the nucleus accumbens which plays a big role as a reward centre. Many studies are performed, focussing on different kinds of epigenetic modifications and transcription factors and their target genes that are involved. It appears that chronic use of cocaine induced modifications in epigenetic pathways that lead to an altered gene transcription. This can be through acetylation of histones (H3 and H4), activation or repressive methylation of histones (H3K9, H3S10) or altered activity of non-coding RNA. This article will give an overview of the basis of addiction and cocaine-induced epigenetic modifications in the nucleus accumbens according to recent studies.

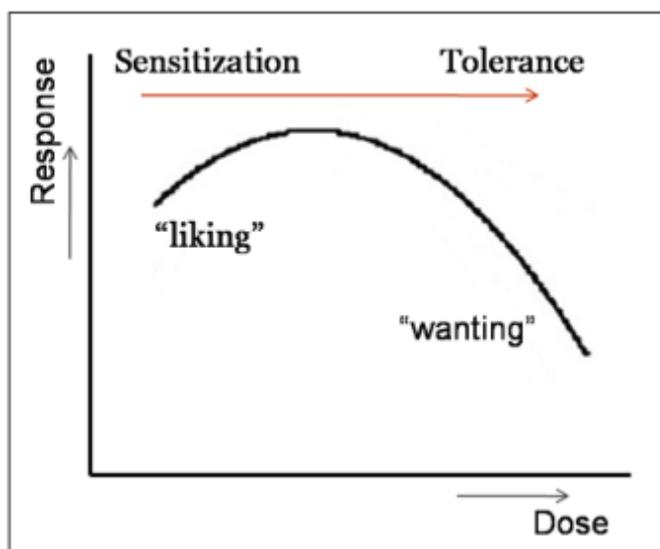


Figure 1. Initially drugs are taken for their pleasurable effect, at which point the brain is still very sensitive to these effects. After repeated use, sensitivity to the drug will decrease requiring higher doses to reach a pleasurable effect. At the point where abnormal high doses are required, the drug is no longer taken for its pleasurable effects, but because the user is dependent on the drug just to feel normal and satisfied. (Anton Scheurink, Lecture Psychobiologie, RUG 2014).

Introduction

Addiction is a worldwide problem and one that is difficult to attack. Many people are victim of a sort of addiction and getting rid of that addiction is never an easy task. This dependence or unwanted abuse can lead to serious problems concerning one's health, social life and finances. Addictions can arise in relation to many different substances and forms of behaviour. Drugs are a large group of substances that lead to addiction. The ones most used are alcohol, nicotine, marijuana, cocaine, heroin, tranquilizers, pain relievers and stimulants. Activities which are famous for causing addictions are gambling, videogames, eating and sexual activities. The most commonly used substance, yet not leading to much trouble, is nicotine or

tobacco. In 2012, 57.5 million Americans of 12 years or older smoked on a regular basis. Alcohol is the drug which accounts for most drug-related problems and the highest number of dependent users with 17.7 million Americans being dependent on the substance or experiencing abuse, reported in 2012. After alcohol, marijuana accounts for the most cases of addiction of all illicit drugs with 4.3 million dependent users in USA in 2012. Also high on the list are pain relievers (2 million), cocaine (1.1 million), tranquilizers (629.000), stimulants (535.000) and heroin (467.000) as reported by the National Institute of Drug Abuse (NIDA) in USA in 2012.

Tolerance

Addiction is a dependence on intake of certain substances or performance of activities that the person needs in order to feel satisfied. Although at first uses intake is voluntary and controllable, it may be completely the opposite once the user has gotten used to the drug, due to tolerance effects. Initially drugs are taken to experience pleasurable effects. Once addicted, drugs are mostly taken just to feel normal again, because the brain has accustomed to large intake of the drug (Figure 1). People feel intense cravings for a drug and even if they really want to quit, they will find that this can be a very large struggle. This is because repeated use of drugs causes changes in neural circuits in the brain which lead to tolerance, due to a shift of the dose-response curve to the right (Figure 2). When tolerance shows, the drug which has been taken in abundance no longer causes the desired effect that one hopes for and a higher dose is required to re-establish those effects. This is because conditioning causes the brain to set a counter reaction in preparation to the drug, which counters the effects of the drug as explained by Neil Carlson in his book *Physiology of Behaviour*. In order to overcome that counter reaction, higher doses are needed which increase the danger of overdosing and causing serious harm to the user.

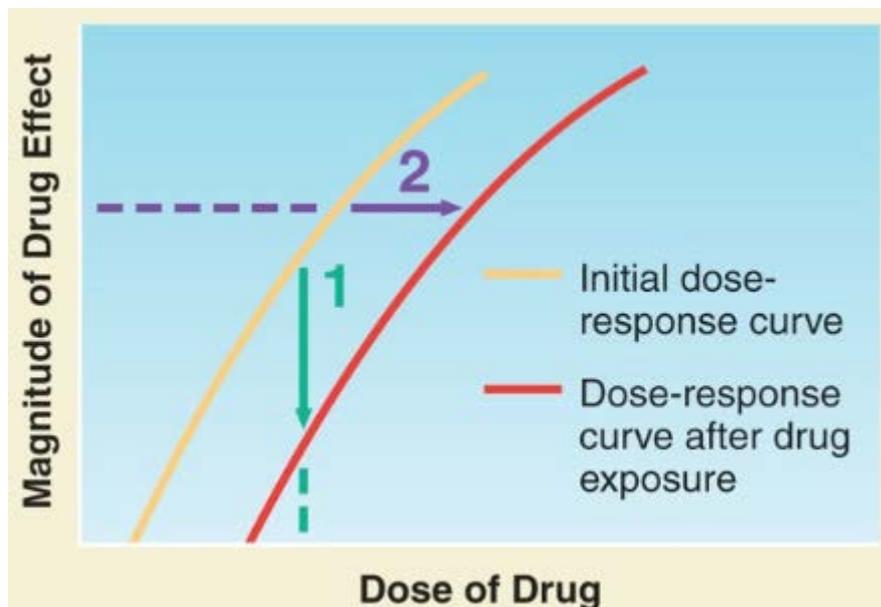


Figure 2. Dose response curve. Tolerance shows after repeated use. Higher doses are required to reach a desired effect, due to decreased sensitivity of the brain. (Pinel 8th, *Biopsychology*, fig 15-3).

The conditioning effect that is mentioned here appears when stimuli associated with drug taking cause the brain to react against the drug even when it is not being used, as explained by Neil Carlson. It is this same conditioning effect that is related to withdrawal symptoms when an addicted user is put off the drug for some time. The compensatory reaction of the brain against the primary effects of the drug appears even when no drugs are consumed (Figure 3). This results in a large craving for the drug to reduce this compensatory reaction. So at first the brain reacts to the effects of

the drug and acts to oppose those effects, but after tolerance the user needs to take drugs to oppose the compensatory reaction.

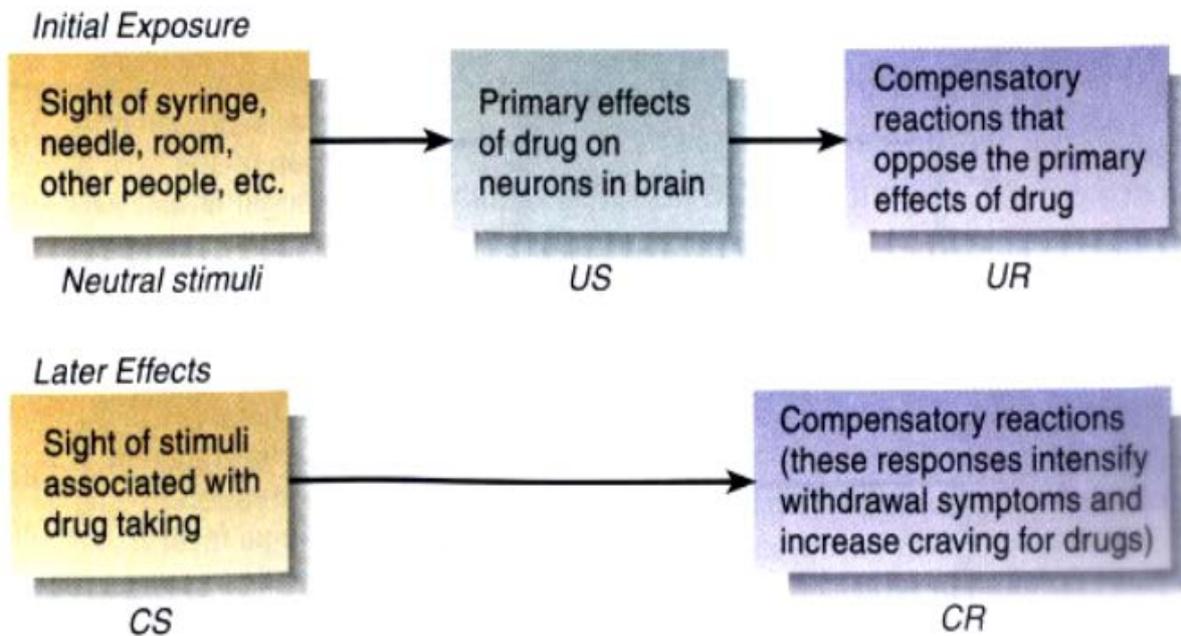


Figure 3. Conditioning effects of drug taking. At first uses it is the drug itself which binds to receptors in the brain and initiates specific reactions. The brain in response will create a compensatory reaction which opposes the effects of the drug. After a while, the user gets conditioned to certain stimuli associated with this drug and these stimuli will yield the compensatory reaction in the brain, even when the drug is not consumed. This compensatory reaction results in large craving for the drug, which is needed to reduce the compensatory reaction of the brain. (Carlson, *Physiology of Behaviour, Classical Conditioning*).

Brain regions associated with addiction

The reason that drug users keep using the drug and become addicted to it is because of the drug's reinforcing effect. And the quicker the drug's effect appears, the stronger this reinforcement effect will be. If the pleasurable feeling arrives quickly, the user will certainly associate this with consuming the drug just shortly before and this association will strengthen on next use (Physiology of Behaviour 11th, N. Carlson). One effect that all addictive drugs have in common is that they cause a release of dopamine in the nucleus accumbens (White, 1996). This is not the only effect mediated by addictive drugs, but it definitely appears to be a necessary step for positive reinforcement to take place (Salamon, 1992). Dopamine is a catecholamine and is thought to have important functions regarding movement, attention, learning and reinforcing effects of drugs (B. Setlow (1997), Physiology of Behaviour 11th, N. Carlson). The most important dopaminergic neurons originate in the substantia nigra (nigrostriatal system) and the ventral tegmental area (mesolimbic system) in the midbrain. Neurons of the nigrostriatal system project to the neostriatum (movement) and neurons of the mesolimbic system project to the limbic system, including the nucleus accumbens, amygdala and hippocampus (Figure 4).

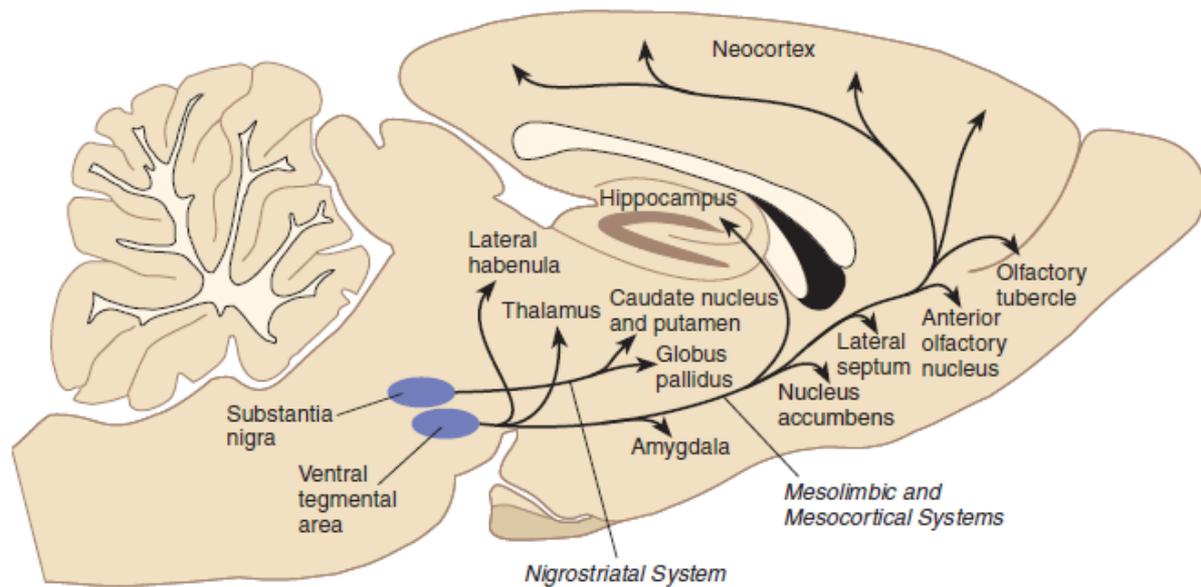


Figure 4. Dopaminergic pathways in a rat brain. Cell bodies of the nigrostriatal system are located in the substantia nigra and project to the neostriatum including the caudate nucleus, putamen and globus pallidus. Cell bodies of the mesolimbic system are located in the ventral tegmental area (VTA) and project to the nucleus accumbens, amygdala and hippocampus. Cell bodies of the mesocortical system are also located in the VTA and project to the prefrontal cortex (Fuxe et al., 1985).

The nucleus accumbens is a region highly associated with forming reinforcing effects of (addictive) drugs. Addictive drugs induce a release of dopamine in the NAc, stimulated by the ventral tegmental area (VTA), which is measured by microdialysis (Di Chiarra, 1995). It is the mesolimbic dopaminergic system where the process of addiction originates. Neurons of this system project to other regions where they subsequently conduct long term changes (Kauer and Malenka, 2007). First changes appear in the VTA where addictive drugs strengthen excitatory synapses of dopaminergic neurons in this area (Saal et al., 2003). These changes come from additional AMPA receptors that appear in the postsynaptic membrane of the dopaminergic neurons (Mameli et al., 2009). As a result of certain changes in the VTA, regions that receive input from this area are observed to have increased their activity. Higher release of dopamine in the nucleus accumbens (NAc) leads to addiction and higher release of dopamine in the dorsal striatum leads to the habit of drug-taking (Volkow et al., 2011). The VTA and NAc are known to play an important role in the reward circuit of drug-taking and changes in these areas may therefore be of great importance (Wise et al., 1992). Findings have already shown that abusive drug-taking increases dopamine receptor mRNA expression in the NAc (Segal et al., 1997). Other changes are increased formation of cAMP and PKA in the NAc. Stimulation of PKA in this area can be seen as a counter reaction against the rewarding properties of cocaine and therefore suggests a tolerance mechanism (Carlezon et al., 1998). Recent interests include the idea of epigenetic changes in the NAc to be the main source of changes in the NAc on a larger scale, such as an increase of synaptic receptors and changes in intracellular pathways and mRNA expression. In this article I will focus on the epigenetics of addiction and how abusive use of drugs can mediate epigenetic changes which may lead to addiction. First I will briefly explain the basics of epigenetics on their own before relating them to addiction.

Epigenetics

Epigenetics can be described as the bridge between genotype and phenotype. It is the study of processes that affect the final outcome of a locus or chromosome without changing the underlying DNA sequence (Goldberg et al., 2007). More specifically, it is the study of stable and heritable changes in gene expression that do not affect the original DNA sequence, therefore keeping it unchanged and intact. The majority of the cells in our body share the same genotype, yet there exists a large variety of differentiated cell types with distinct cellular functions. This differentiation can be attributed to epigenetics. Research focuses on modifications of DNA and histone proteins and the mechanisms that lie behind these modifications which influence the chromatin structure. There are several different types of modifications (Figure 5). The two main mechanisms that cause chromatin remodelling are DNA methylation and histone modification (R. Holliday, 2006).

DNA methylation is currently the best characterised modification of chromatin structure. DNA methylation normally occurs on the cytosine residue of CpG dinucleotides (Holliday, 1994). Regions with high density of CpG dinucleotides are also known as CpG islands. Methylation at these regions is associated with transcriptional silencing and gene regulation, which is performed by methyltransferases that add methyl groups to DNA (Goldberg et al., 2007). Methyl groups bound to the DNA sequence silence this area and prevent transcription. In addition to DNA methylation, there are histone modifications. The best studied type is histone acetylation. Chromatin can be in open form or closed. Only an open form allows for DNA transcription. Histone acetylation plays an important role in the formation of chromatin. When histones are acetylated, chromatin is found in its open form, allowing DNA transcription. Hypoacetylated or deacetylated histones are associated with closed chromatin, associated with repression of DNA transcription (Zaratiegui et al., 2007).

Recently research has focused on the fact that addictive drugs may induce epigenetic changes in the brain which alter gene expression and therefore mediate an addictive state. In the next part of this article, the relation of epigenetics and addiction will be introduced and several studies on epigenetic changes induced by drugs will be discussed.

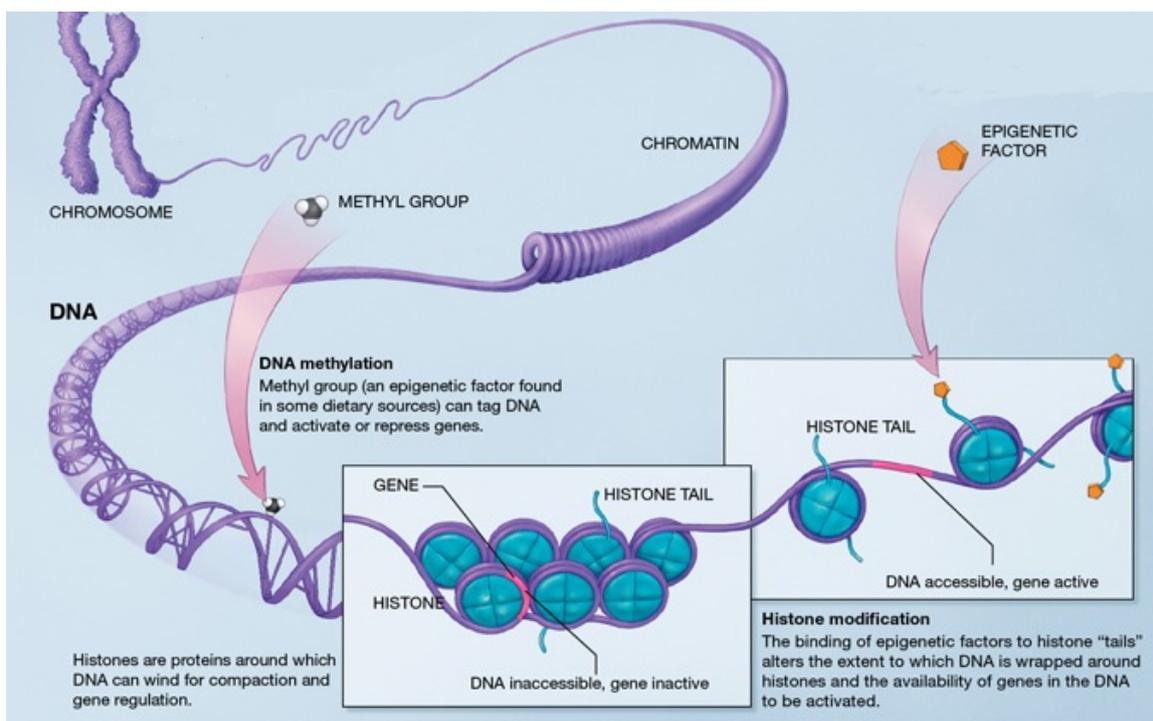


Figure 5. Overview of epigenetic modifications. Binding of a methyl group to the DNA can silence that region and prevent transcription of genes. Binding of epigenetic factors such as acetyl groups opens up the DNA that is wrapped around histones and allows for transcription (National Institutes of Health, Office of Strategic Coordination, The Common Fund).

Epigenetics and relation to addiction

Some drug users are very prone to becoming addicted, while others may be able to take drugs and get away without the risk of developing an addictive state. This is because genetic factors make up about 50% of all factors that contribute to the vulnerability of developing dependence to the drug (Nestler, 2013). Studies on candidate genes have identified several genes whose expression is altered in mouse models of addiction, but also in human addicts. In addition, transcription factors that regulate transcription of genes are being associated with long term effects of drug use on gene expression. Recent studies (Feng et al., 2014) suggest that epigenetic regulation, such as DNA methylation, may be responsible for alterations in the brain as a consequence of abusive usage of addictive drugs (Figure 6). Modified histones induce typical drug-related behaviour, also after refraining from that drug for a long time which suggests long term and stable changes (Renthal & Nestler, 2008). This also explains why users who have overcome their addiction can drop right back into it after only one encounter with the drug that started it all. Therefore, the adaptations induced by abusive drug use can be seen as cellular memory (Nestler, 2013). What is interesting is that most of the types of changes associated with addiction have also been observed in traditional behavioural memory processes, once more indicating that drugs lead to memory formation by long term changes in responsible regions in the brain, such as the VTA and NAc. Next, findings from recent studies on epigenetic changes induced by cocaine will be shown and discussed. These include altered epigenetic modifications and the genes affected by these modifications.

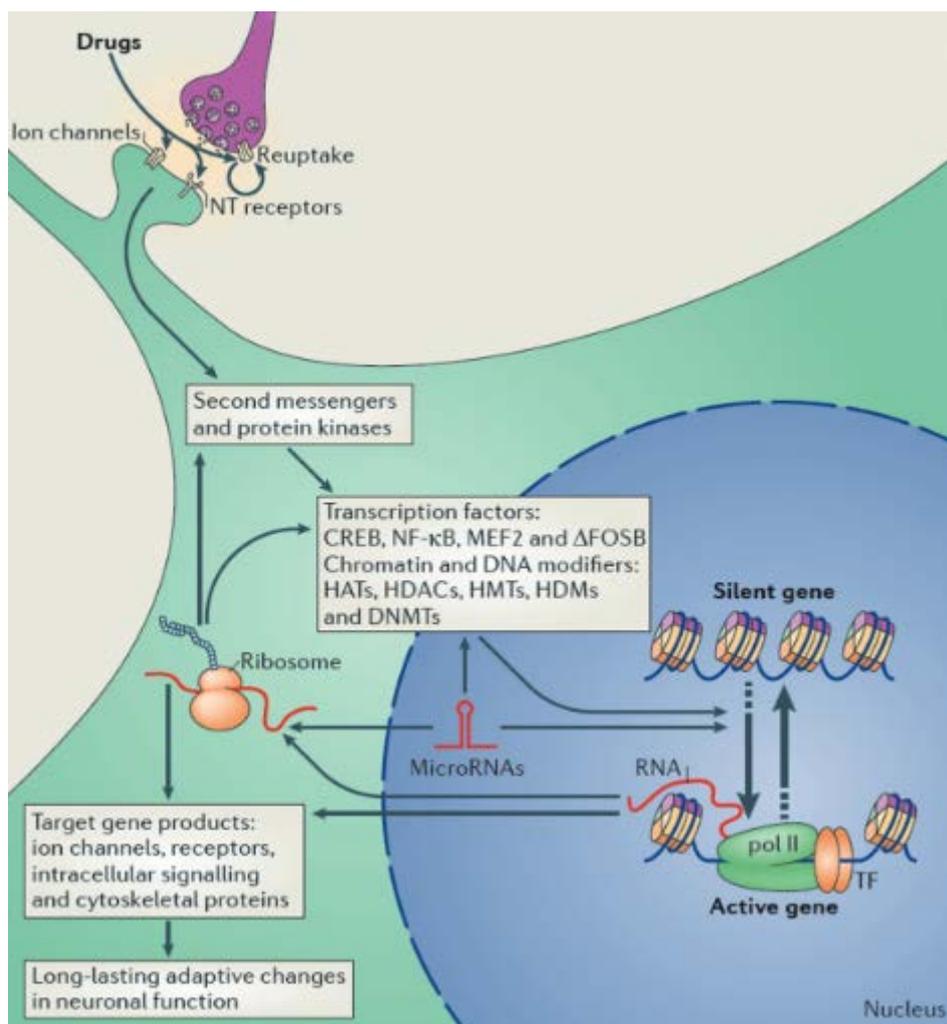
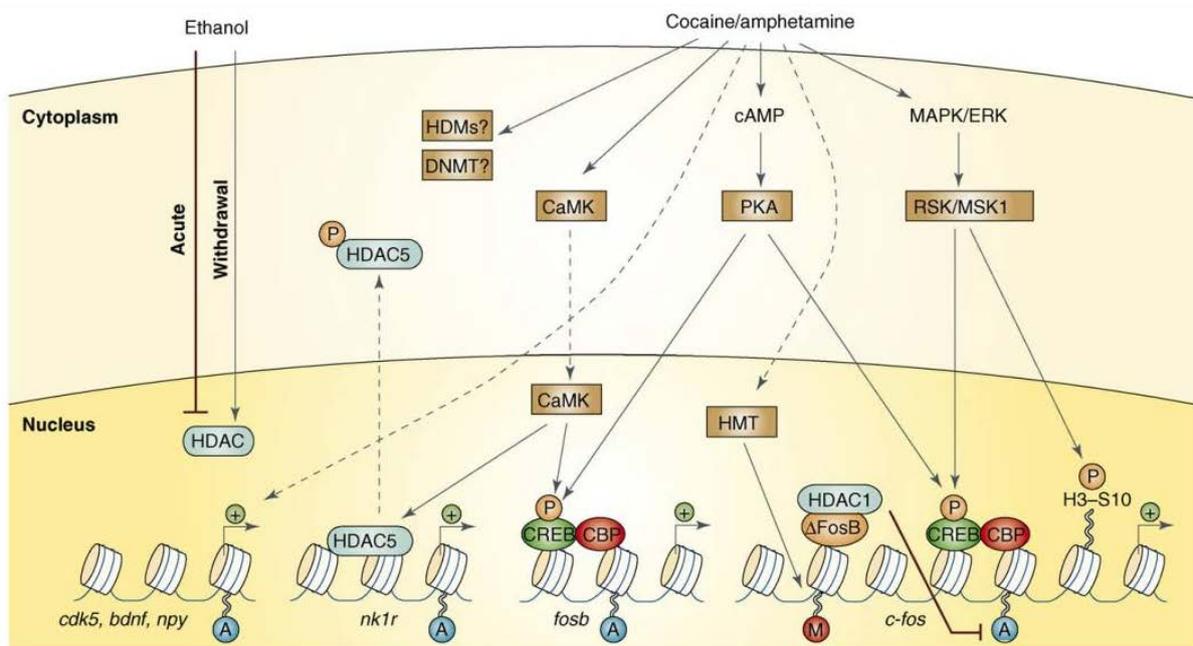


Figure 6. Mechanism of epigenetic regulation by drugs. Drugs target at the synapse where they mediate release of neurotransmitters, re-uptake mechanisms or blockage of post-synaptic receptors. This either activates or inhibits an intracellular pathway leading to activation or inhibition of transcription factors and other regulatory proteins. This eventually leads to an altered gene expression, increased or decreased. (Robison & Nestler, 2011).

Effects of cocaine consumption on histone modification

Acute and chronic effects of cocaine on histone H3 and H4

Both acute and chronic intake of cocaine creates increased activation of specific genes, but only long-term intake leaves behind long-term changes in gene regulation (Bilinski et al., 2012). Some genes become highly active, but others may soon drop to levels even below their usual activity due to desensitisation. This desensitisation is a change induced by cocaine intake which is partially responsible for higher doses being consumed and a craving for the drug. This effect can be attributed to the modifications made to histone H3 and H4 in the reward centre (Cheung et al., 2000; Renthal & Nestler, 2008). Histones are made up of 4 different histone proteins, H2A, H2B, H3 and H4. When a first ever dose of cocaine is administered, chromatin become more densely packed and therefore reduce gene transcription. This is mediated by a hyper-activation of histone methyltransferases (HMT) and histone deacetylase (HDAC), which both prevent transcription factors from binding to a specific region of the DNA and therefore repressing that region, by modifying histone H3 and H4 (Kumar et al., 2005). But when cocaine is chronically applied, the opposite is observed. Acetylation of histones is found in high amounts while methylation has decreased as a consequence of activation and inhibition of the respective enzymes and an increase of the Δ FosB and CREB transcription factors. Δ FosB and CREB (cAMP-response-element-binding protein) are involved with the activation of genes that induce neuronal plasticity in the NAc which is important for developing addiction (Nestler, 2001). Chronic cocaine induced hyper-acetylation on the H3 histone which affected genes involved with neuronal plasticity in the NAc, such as CDK5 (Cyclin Dependent Kinase 5), BDNF (Brain-Derived Neurotrophic Factor) or NPY (Neuropeptide Y) (Kumar et al., 2005). More detail on the CDK5 pathway will follow later. In contrast to hyper-acetylation of the H3 histone after chronic treatment of cocaine, the H4 histone was hyper-acetylated after acute treatment.

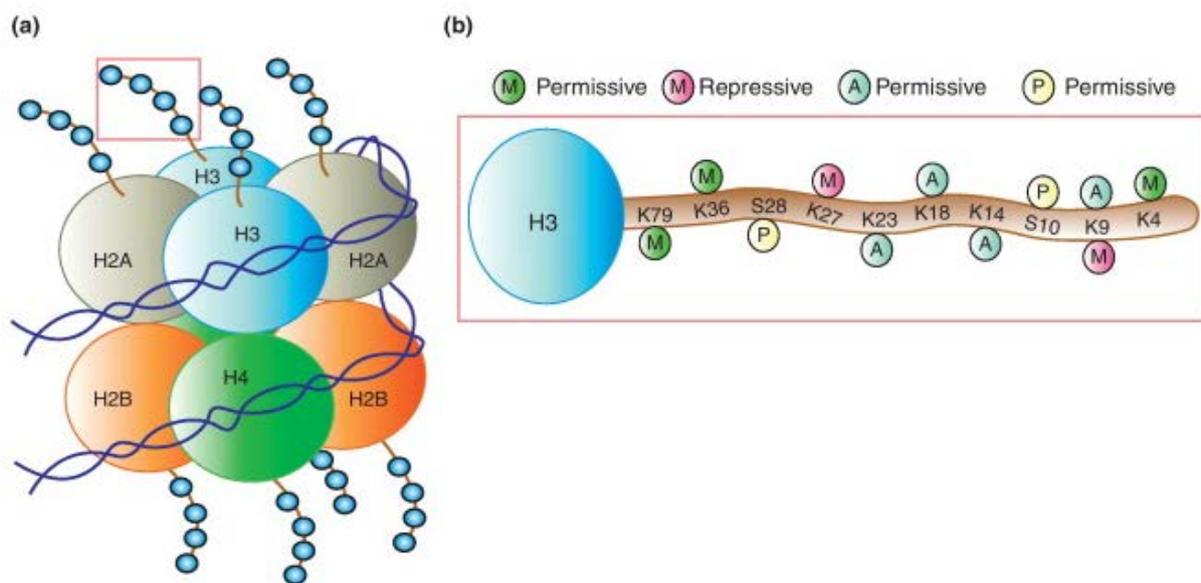


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Figure 7. Effects of cocaine (and amphetamine) on chromatin remodeling. Binding of cocaine increases cAMP levels, which increases PKA. PKA phosphorylates CREB, which allows recruitment of CBP (CREB binding protein). Gene transcription is now possible on genes such as *fosb* or *c-fos*. Chronic use of cocaine can affect transcription of *CDK5*, *BDNF* and *NPY* genes directly (Tsankova et al., 2007).

Histone methylation

Histone methylation can occur on lysines 9, 14, 18 and 23 on histone H3 and on lysines 5, 8, 12 and 16 on histone H4. Methylation of H3 lysine 9 (H3K9) may be the best characterised, because it reduces the activating pathway induced by phosphorylation of histone H3 serine 10 (H3S10), which is the best characterised type of histone phosphorylation (Maze & Nestler, 2011). Methylation of histones can either result in initiation or repression of transcription (Figure 8). Tri-methylation of H3K4 and H3K36 are associated with increased levels of transcription. In contrast, di- and tri-methylation of H3K9 and H3K27 are associated with repression of gene transcription (Rice & Allis, 2001). Chronic cocaine use reduces the methylation of H3K9 in the NAc (Bilinski et al., 2012). This is mediated by inhibiting the G9A gene which codes for a histone dimethyltransferase. In addition, there is the increase of Δ FosB as a consequence of chronic cocaine use, which also reduces H3 methylation. As mentioned before, this supports neuronal plasticity by an increase in neuronal dendrites in the NAc and forming new synaptic connections in the NAc and therefore boosting the reward centre when cocaine is consumed. These new connections are stable and will last for a long time, so that is a good example of how a combination of epigenetic changes and increase of the Δ FosB transcription factor, both induced by cocaine, increases the reward received from taking the drug.



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Figure 8. Modifications of the H3 histone protein. A) General structure of a histone, consisting of 2 copies of H2A, H2B, H3 and H4. B) Different types of modifications that are possible on the H3 protein. Methylation of the K9 lysine represses transcription. It is known that cocaine reduces methylation of H3K9 and therefore stimulates transcription. Through this process, transcription of Δ FosB is allowed which increases neuronal plasticity by forming new synapses in the NAc which increases the reward received from cocaine (Feng & Nestler, 2013).

Tri- or dimethylated H3K9 is one of the best markers for recognizing heterochromatin. It was found that repeated use of cocaine actually unsilences specific regions that were previously silenced (Maze et al., 2011). The interesting thing is that specific chromatin modifying enzymes are not affected by cocaine use, but several histone and DNA enzymes, such as G9A and DNA methyltransferases (DNMTs) are regulated by exposure to cocaine. As mentioned before, methylation of H3K9 is

mediated by a complex including the G9A enzyme and it is this complex that is affected. (Maze et al., 2010). G9A and GLP (G9A-like protein) catalyze the dimethylation of H3K9 (H3K9me2) and when cocaine causes a downregulation of these enzymes, a decrease in H3K9me2 is observed. G9A mRNA expression was reduced by 35% in the NAc, accompanied by a 15% decrease in G9A protein levels and a 21% decrease in H3K9me2 after repeated treatment with cocaine (Figure 9a). Looking at upstream processes that may mediate this reduction in G9A mRNA expression, it is found that Δ FosB plays an important role here. Δ FosB is found in high levels in the NAc after repeated cocaine administration. Before, I have already mentioned the important role of this product in the increased reward for cocaine (Nestler, 2008). Overexpression of Δ FosB decreased levels of H3K9me2 and expression of G9A in the NAc (Figure 9b, 9c). In order to confirm the effects of Δ FosB on G9A expression alone, mice were received injections into the NAc containing an Adeno-associated virus (AAV) expressing either GFP or Δ FosB. Mice injected with AAV- Δ FosB showed reduced levels of G9A expression in the NAc region, in contrast with the AAV-GFP (control) group which showed unaffected levels of G9A expression (Figure 9c). It has been shown that these effects are mediated by repeated administration of cocaine. Although for a long time the cellular mechanisms of addiction to other substances such as opiates remained largely unexplored. However, recently it has been discovered that downregulation of G9a and H3K9me2 in the NAc also holds true for administration of morphine (Sun et al., 2012).

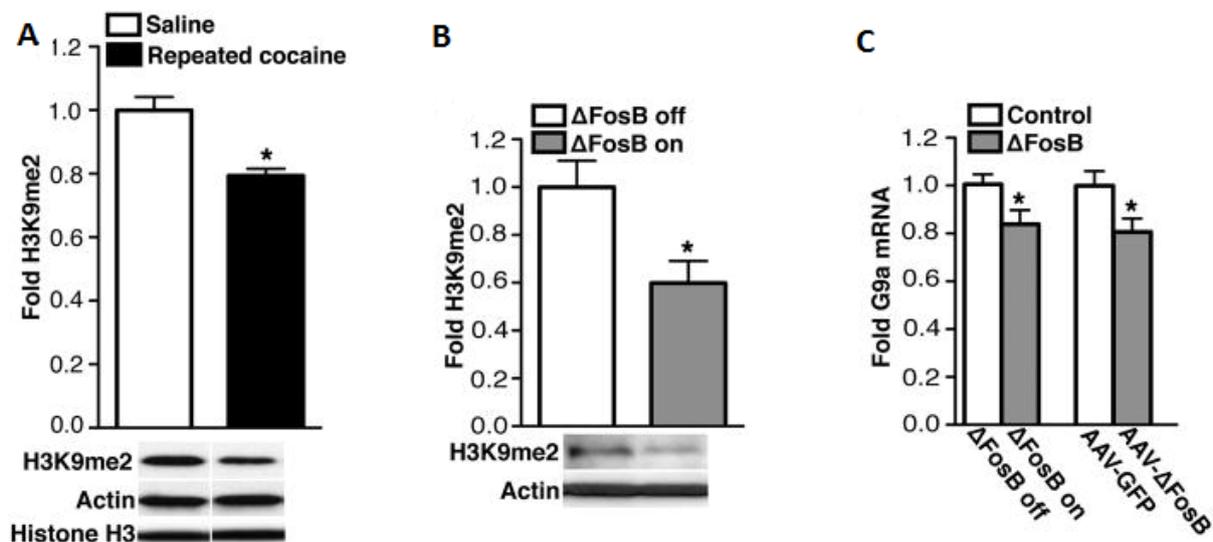


Figure 9. Cocaine alters G9A mRNA expression levels by increasing levels of Δ FosB in the NAc. **A)** H3K9me2 after 24 hours of repeated cocaine administration. Repeated cocaine lowers H3K9me2 levels. This is explained by: **B)** Increased levels of Δ FosB decrease H3K9me2 levels as measured using a specific Δ FosB on/off mouse model. **C)** Mice injected with a virus containing a Δ FosB vector showed decreased expression of G9A mRNA. Since G9A is involved with methylation of H3K9, this explains the results of graph B (Maze et al., 2010).

Δ FosB induces CDK5

It has become very clear that Δ FosB plays an important role in the process of developing changes in gene regulation in the NAc and developing an addictive state. Δ FosB regulates these effects by inducing specific genes. One gene in particular is CDK5 (E. Nestler, 2008). Δ FosB binds to this gene and then recruits histone acetyltransferases. These acetylate histones after which gene transcription of CDK5 is allowed. CDK5 is important because it is directly linked to the

phosphorylation of several synaptic proteins (Bibb et al., 2001) and is also associated with an increase in dendritic spine density (Norrholm et al., 2003). Chronic cocaine use can lead to increases in dendritic spine density in the NAc through activation of Δ FosB and CDK5 and thereby increase neuronal plasticity and cellular memory. It is thought that normally induction of moderate levels of Δ FosB by rewarding stimuli, such as addictive drugs, would be adaptive for the user. This would mean that the user adjusts to increased levels. However, when excessive levels of Δ FosB are given rise to by chronic administration of abusive drugs, excessive sensitization can occur in the NAc. This could lead to pathological behaviour such as compulsive drug seeking and taking, which can be seen in drug addicts (Nestler, 2008).

Reduced H3K9 methylation increases vulnerability to stress

Methylation of H3K9 has also been linked to increased stress vulnerability. It was already known that a mood disorder, such as stress, could increase the chance of developing substance abuse. However, the opposite always remained unexplored. It was found that repeated cocaine administration in combination with chronic social defeat stress could give rise to developing depressive-like behaviours in mice through decreased methylation (Figure 10) of H3K9 by reduction of the G9A and GLP methyltransferases complex (Covington et al., 2011). G9a knockdown in the NAc of mice, promoted increased vulnerability to social stress. Since cocaine reduces G9a activity, cocaine could also lead to stress-like behaviours. However, overexpression of G9a in mice prevented susceptibility to social stress after repeated cocaine treatment, in contrast with mice who did not receive overexpression of G9a.

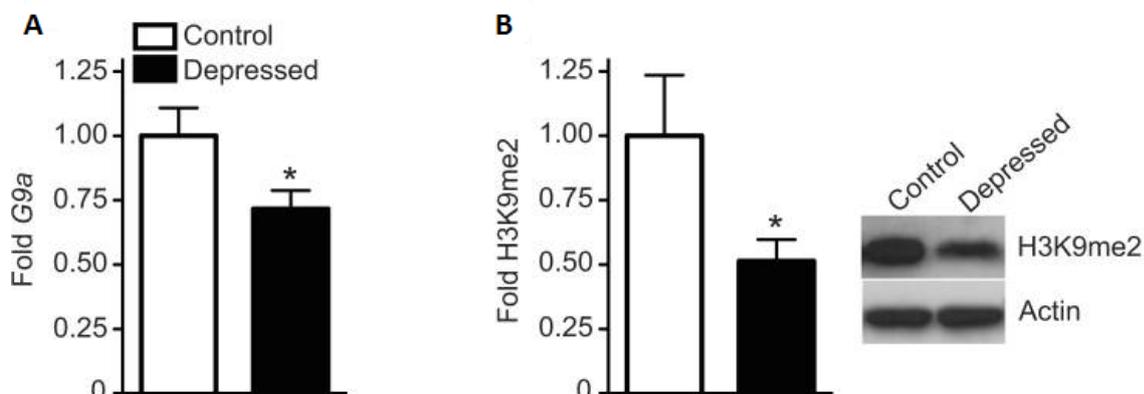


Figure 10. G9a and H3K9me2 levels in control and depressed mice. Levels of G9a and H3K9me2 are both lowered in depressed mice, which is an effect that also comes with repeated cocaine use. This suggests a possible link between cocaine use and depressive-like behavior, through increased vulnerability to social defeat stress (Covington et al., 2011).

CREB transcription factor

Researchers have also found an important role of BDNF-TrkB-CREB signalling, which is associated with addiction- and depression-related processes in the NAc. Chronic administration of cocaine induced a RAS signalling pathway, where RAS activates BDNF-TrkB signalling which in turn activates CREB and through this pathway increases the vulnerability to social stress. Once again, overexpression of G9a reduces increases in RAS activity in the NAc after repeated cocaine use and also reduces phosphorylation of all downstream components of the signalling cascade (Covington et al., 2011). CREB is an important transcription factor associated with the formation of long-term

memories in the brain, which may lead to drug addiction when abusive drugs stimulate the CREB pathway (Nestler, 2013). CREB is involved in a negative feedback system when activated by addictive drugs and its activation reduces the user's sensitivity to the rewarding effects of the drug. (Nestler, 2012). This leads to drug tolerance and withdrawal effects when the user refrains from the drug. However, CREB activation still stimulates drug taking, regardless of the reduced reward. Medium spiny neurons in the NAc are involved in this process of forming long-term memories for the rewarding effects of addictive drugs, by increased excitability of these medium spiny neurons after CREB overexpression. Previous research has already shown that CREB is an important factor in behavioural memory (Kandel, 2012). Target genes mediating addictive effects include the opioid peptide dynorphin, which suppresses dopaminergic signalling from the VTA to the NAc as a type of feedback system (Carlezon et al., 1998). Another target is the GluA1 AMPA and GluN2B NMDA subunit. Together these two targets are thought to be involved with increased excitability of medium spiny neurons after CREB overexpression (Dong et al., 2006). Another transcription factor that is induced by chronic cocaine administration is NFκB (Ang et al., 2001). NFκB is also involved with formation of dendritic spines on medium spiny neurons in the NAc. However, its target genes are currently not well documented.

Effects of cocaine use on DNA methylation

In contrast to epigenetic regulation by histone modification, little is known about the precise role of DNA methylation in the development of addiction to substances such as cocaine. Research has shown that altered levels of DNA methylation in the hippocampus may be important for drug-associated changes in gene expression. Following cocaine administration to mice, DNA methylation

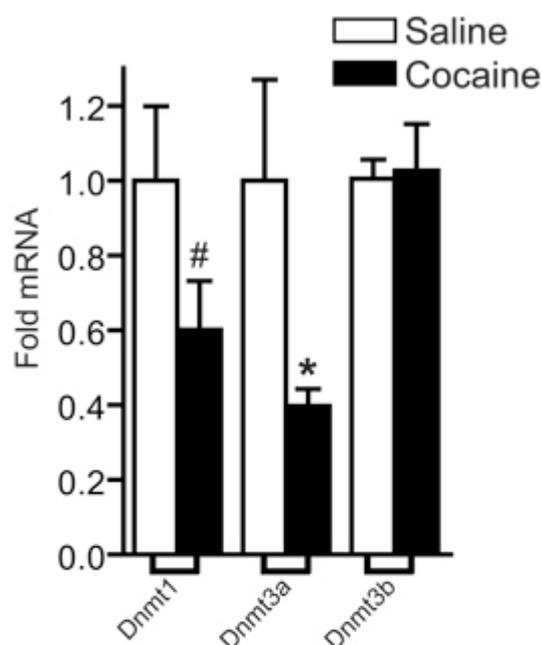


Figure 11. Levels of mRNA of DNMT1, DNMT3a and DNMT3b, 24 hours after the last cocaine injection. Expression of DNMT3a was significantly decreased by chronic cocaine administration. Alterations in expression of DNMT1 and DNMT3b were not significant (Q. LaPlant et al., 2010).

was significantly reduced 3 days post-administration, but increased 30 days post-administration (Novikova et al., 2008). Other studies also showed that methyl CPG binding protein 2 (MeCP2) was induced in cortical regions and the striatum after chronic cocaine use (Anier et al., 2010), which correlated with increased expression of the MEF2C transcription factor (Host et al., 2011). More importantly, an important role for the DNA methylating enzyme DNA methyltransferase 3a (DNMT3a) has been found. Chronic administration of cocaine caused a downregulation of DNMT3a expression 24 hours after the last injection (Figure 11) and it also caused increased behavioural responses (LaPlant et al., 2010). This makes sense, since DNMT3a is an inhibiting factor for gene transcription and reducing it should increase gene expression which correlates with the increased responses that were found. This study also showed that overexpression of DNMT3a increased spine density in the NAc, even in absence of cocaine which

is already known to increase spine density. These same effects are mediated by CREB, which suggest a possible link between DNMT3a and CREB.

Effects of cocaine use on non-coding RNA

Lastly, there is another way of regulating gene expression without modifying nucleotide sequence and that is mediated by non-coding RNA. The most important and best studied non-coding RNA regulating mechanism is gene regulation by micro RNAs (miRNAs). miRNAs bind to mRNAs and repress their expression by preventing translation by ribosomes (Feng & Nestler, 2013). Studies have shown that cocaine up- or downregulates specific miRNAs. It increases levels of miR-181a and decreases miR-124 and let-7d in the rat striatum (Chandrasekar & Dreyer, 2009). Gain and loss of function of above miRNAs on cocaine-induced conditioned place preference (CPP) was tested by using lentiviral vectors (LV) expressing these specific miRNAs. LV-miR-124 and LV-let-7d expression in the NAc reduced CPP induced by cocaine, but LV-miR-181a increased it (Chandrasekar & Dreyer, 2011). Silencing these specific miRNAs by using LV-miRNA silencers reversed these effects. These studies suggest that miRNAs may be involved in a complex regulatory pathway, induced by cocaine administration. Another study has found that miR-212 may inhibit cocaine intake, after first being induced by cocaine administration in rats (Hollander et al., 2010). This miRNA is expressed in the striatum and is upregulated by cocaine. miR-212 inhibits intake of cocaine by stimulating the CREB signalling pathway, a transcription factor that reduces cocaine reward (Nestler, 2012), as described before in this article. Detailed information regarding the involvement of non-coding RNAs on the effects of cocaine remains limited, but recent research definitely suggests a significant contribution by these gene expression regulatory factors.

Discussion and future directions

From many studies performed over recent years, it has become clear that epigenetics play a very important role in the development of addiction to cocaine. Epigenetic mechanisms are affected by cocaine administration and change into different mechanisms that promote an addictive state. The most important of epigenetic changes seems to be histone modifications. H3K9 dimethylation is an example of a major component which is an intensively studied histone methylation receiving plenty of attention recently. Not only do epigenetic modifications appear to be an important factor in developing addiction, but they may even be the main cause of addiction once a drug like cocaine affects the functioning of these mechanisms in the NAc. This could then also be the underlying factor for typical behaviour such as drug craving, drug seeking, withdrawal symptoms and emotional states that accompany these behaviours. The finding that epigenetics have such an important influence is of great importance for the development of new therapies and medication. Of course the effects of cocaine still need to be determined more precisely, but that is only a matter of time. If the right mechanisms are targeted, this could prevent epigenetic changes from leading to an addictive state. Other therapies that are also highly needed are ways to prevent withdrawal symptoms from occurring in order to increase the chance of success of quitting. Users are often victim to the large craving for the drug and will find it hard to fully escape from their addiction. If in a way this craving

for the drug and the urge to experience its effects could be decreased or even fully prevented, that would be a huge step forward.

A recent study has looked at the affectivity of histone deacetylase (HDAC) inhibitors on epigenetic modifications induced by cocaine in the NAc of the mouse brain (Kennedy et al., 2013). They found that as a result of prolonged class 1 HDAC inhibitor administration, blocking HDAC1 in the NAc, increased levels of histone acetylation in the area. In addition this HDAC inhibitor also increased histone methylation which repressed gene transcription. Through these mechanisms, cocaine-induced effects were blocked and so gene alterations by cocaine could not take place. H3K9 methylation was increased, in contrast to decreased H3K9 methylation after chronic cocaine use. Since reduced H3K9 methylation is involved in neuronal plasticity after cocaine use, increasing this process counter affects the cocaine-induced changes. This suggests a good potential for administration of this drug against addiction in the future. However, most research on this topic focuses on brain areas such as the VTA and NAc. It is important that we also study other regions of the brain such as a possible involvement of the hippocampus in addiction memory. This doesn't mean that the main centre for addiction isn't found in the VTA or NAc, but there can always be crosstalk between these and other areas. Lastly, it is important to discover more transcription factors and to find out exactly how they activate or block their target genes. Δ FosB is an important transcription factor involved with neuronal plasticity after chronic cocaine use, so it is crucial to understand exactly how the associated pathway is set up in order to block it effectively. Since new information is constantly being discovered, one day we will have sufficient knowledge to start developing medication targeting epigenetic and transcriptional factors. Current therapies don't seem sufficient enough to cure all addicted patients and it looks like epigenetics could promise a bright future for those people.

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