Essential Role of the Histone Methyltransferase G9a in Cocaine-Induced Plasticity
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of cocaine.

Using conditional mutagenesis and viral-mediated gene transfer, we found that G9a down-regulation of G9a in this brain region, which was regulated by the cocaine-induced transcription factor ∆FosB. This reduction in histone methylation was mediated through the repression of neonatal gene expression of both genes was significantly down-regulated (4, 7, 28), the PC niche is a holding pen, which does not allow its cells to escape or to differentiate until the niche breaks down. The transition to a functionally homeostatic adult niche that maintains ISCs would require a separate step. Our observations indicate a paradigm that other stem cell systems may also use: The progenitor cell divides to form both niche and stem cells. Such a mechanism that lends greater autonomy to stem cells might exist in other epithelial cell populations during development or tissue homeostasis.

References and Notes

Essential Role of the Histone Methyltransferase G9a in Cocaine-Induced Plasticity

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Cocaine-induced alterations in gene expression cause changes in neuronal morphology and behavior that may underlie cocaine addiction. In mice, we identified an essential role for histone 3 lysine 9 (H3K9) dimethylation and the lysine dimethyltransferase G9a in cocaine-induced structural and behavioral plasticity. Repeated cocaine administration reduced global levels of H3K9 dimethylation in the nucleus accumbens. This reduction in histone methylation was mediated through the repression of G9a in this brain region, which was regulated by the cocaine-induced transcription factor ΔFosB. Using conditional mutagenesis and viral-mediated gene transfer, we found that G9a down-regulation increased the dendritic spine plasticity of nucleus accumbens neurons and enhanced the preference for cocaine, thereby establishing a crucial role for histone methylation in the long-term actions of cocaine.

Repeated cocaine exposure is characterized by persistent changes in gene expression and altered neuronal morphology within the rodent nucleus accumbens (NAc), a key component of the brain’s reward circuitry (1, 2). Chromatin remodeling is important in aberrant transcriptional changes in this brain region that may underlie aspects of cocaine addiction (3–9). Cocaine regulation of chromatin structure in the NAc results, in part, from direct cocaine-induced modifications of the chromatin enzymatic machinery, leading to changes in histone acetylation and phosphorylation (4, 7–9); however, roles for enzymes controlling histone methylation have not yet been investigated.

A recent genome-wide promoter analysis using chromatin immunoprecipitation coupled to microarrays (ChIP-Chip) identified altered patterns of repressive histone H3 lysine 9 (H3K9) and 27 (H3K27) methylation at specific gene promoters in the NAc after repeated cocaine treatment (6). In mice, we therefore profiled numerous lysine methyltransferases (KMTs) and demethylases (KDMs) that are known to control H3K9 or H3K27 methylation (Fig. 1A). Only two enzymes, G9a and G9a-like protein (GLP), displayed persistent transcriptional regulation 24 hours after repeated cocaine administration, when the expression of both genes was significantly down-regulated. Because G9a and GLP specifically catalyze the dimethylation of H3K9 (H3K9me2), their down-regulation by cocaine is consistent with decreased global levels of euchromatic H3K9me2 observed at this time point (Fig. 1B). In contrast, global levels of heterochromatic H3K27 methylation were increased. Therefore, we investigated the role of G9a in cocaine-induced plasticity in the NAc.

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Fig. 1. Repeated cocaine administration represses G9a expression in the NAc through a ΔFosB-dependent mechanism. (A) mRNA expression of H3K9/K27 KMTs and KDMs in the NAc 24 hours after repeated cocaine. (B) H3K9me2 levels in NAc 24 hours after repeated cocaine. (C) Analysis of gene expression after acute or repeated cocaine. Heat maps (*) show genes up-regulated in NAc 1 hour after a cocaine challenge in naïve animals (acute), in animals treated repeatedly with cocaine (repeated + acute), or in animals after 168 hours of withdrawal from repeated cocaine (repeated wd + acute). Associated heat maps show how genes were affected under the other two conditions. Desensitized transcriptional responses after repeated cocaine are indicated (***). (D) H3K9me2 levels in the NAc from NSE-tTA x tetOP-ΔFosB mice on (ΔFosB off) or off (ΔFosB on) doxycycline 1 hour after repeated cocaine. (E) G9a mRNA expression in the NAc from NSE-tTA x tetOP-ΔFosB mice on (ΔFosB off) and off (ΔFosB on) doxycycline and from mice infected with AAV-GFP or AAV-ΔFosB. Data are presented as mean ± SEM. For statistical analyses, see the full figure legends in the supporting online text.

Fig. 2. G9a in NAc regulates cocaine-induced behavioral plasticity. (A) Representative image of HSV-mediated transgene expression in the NAc. The cartoon of the coronal brain slice was taken from the mouse brain atlas. (B) Conditioned place preference for cocaine and (C) H3K9me2 levels in the NAc in animals infected with HSV-GFP, HSV-G9a, or HSV-G9aH1093K. (D) Conditioned place preference for cocaine and (E) H3K9me2 levels in the NAc in G9a−/− animals infected with AAV-GFP or AAV-Cre. (F) Conditioned place preference for cocaine and (G) H3K9me2 levels in the NAc in animals receiving intra-NAc vehicle or BIX01294. Data are presented as mean ± SEM.
ylation remained unaltered by repeated cocaine exposure (fig. S1). Because of its high levels of catalytic activity both in vitro and in vivo (10), we set out to further investigate the functional significance of G9a repression after repeated cocaine exposure in the NAc. Levels of G9a protein, like levels of its mRNA, were significantly reduced 24 hours after repeated cocaine administration (fig. S2). Although G9a mRNA expression was reduced by 35% in the NAc, immunohistochemical analysis revealed a more modest 15% reduction in G9a protein levels, which is consistent with the observed 21% decrease in H3K9me2 after repeated cocaine administration (Fig. 1B). G9a mRNA expression was also down-regulated in this brain region by 20% after repeated self-administration of cocaine (fig. S3).

To identify whether changes in euchromatic H3K9me2 correlate with genome-wide alterations in gene expression in the NAc, we employed microarray analyses to examine gene expression profiles induced by a challenge dose of cocaine in mice with or without a history of prior cocaine exposure (see the gene lists in tables S1 to S3). Animals that had received repeated cocaine displayed dramatically increased gene expression 1 hour after a cocaine challenge, in comparison to acutely treated animals (Fig. 1C). This increased gene expression still occurred in response to a cocaine challenge given after 1 week of withdrawal from repeated cocaine. Consistent with previous reports, a small percentage of genes (~10%) displayed desensitized transcriptional responses after repeated cocaine administration (Fig. 1C and table S1) (5). To directly investigate the role of G9a down-regulation in the enhanced gene expression observed after repeated cocaine exposure, mice received intra-NAc injections of herpes simplex virus (HSV) vectors expressing either green fluorescent protein (GFP) or G9a and were treated with saline or repeated cocaine to determine whether G9a overexpression was sufficient to block the repeated cocaine-induced enhancement of gene expression. From a set of 12 randomly selected genes displaying heightened levels of expression after repeated cocaine, we observed that G9a significantly reduced the enhanced expression of 50% of these genes (table S4).

To identify upstream transcriptional events that mediate the repeated cocaine–induced repression of G9a expression, we investigated a possible role for ∆FosB, a highly stable splice product of the immediate early gene fosB. ∆FosB accumulates in the NAc after repeated exposure to cocaine, where it has been linked to increased cocaine reward (11). ∆FosB can act as either a transcriptional activator or repressor, depending on the target gene involved (3, 5, 6, 12). Using bi-transgenic NSE–tTA x tetoP–∆FosB mice, wherein ∆FosB expression can be induced selectively in the NAc and dorsal striatum of adult animals (13), we examined the impact of ∆FosB expression on cocaine regulation of H3K9me2 and KMTs in the NAc. ∆FosB overexpression was sufficient to reduce levels of both H3K9me2 (Fig. 1D) and G9a expression (Fig. 1E), thereby mimicking the effects of repeated cocaine. In contrast, ∆FosB did not reduce GLP expression in this brain region and had no effect on SUV39H1 and EZH2, the principal trimethylating enzymes for H3K9 and H3K27, respectively (fig. S4). To confirm these data using an independent ∆FosB overexpression system, wild-type adult mice received bilateral intra-NAc injections of adenoassociated virus (AAV) vectors expressing either GFP or ∆FosB. Viral-mediated overexpression of ∆FosB decreased levels of G9a expression in this brain region (Fig. 1E).

Such pronounced and specific regulation of G9a prompted us to investigate whether altering G9a expression specifically in NAc neurons regulates behavioral responses to cocaine. Wild-type mice received intra-NAc injections of HSV vectors expressing GFP or G9a and were then analyzed with an unbiased cocaine-conditioned place-preference paradigm, which provides an indirect measure of drug reward. Viral overexpression of G9a in NAc neurons was con-

Fig. 3. G9a in the NAc regulates cocaine-induced dendritic spine plasticity. (A) Quantitative G9a ChIP in the NAc from animals treated acutely or repeatedly with cocaine, at 1 or 24 hours, respectively. Adenine phosphoribosyltransferase (APRT) was used as a negative control. Data are presented as the relative fold difference from saline controls. (B) Quantitative H3K9me2 ChIP in the NAc from repeated cocaine–treated animals at 24 hours, presented as the relative fold difference from saline controls. (C) Dendritic spine analysis of animals infected with HSV-GFP, HSV-G9a, or HSV-∆JunD after repeated cocaine, and dendritic spines in G9a−/− mice after HSV-Cre infection. (D) Quantitative G9a ChIP in the NAc from NSE–tTA x tetoP–∆FosB mice on (∆FosB off) and off (∆FosB on) doxycycline. (E) Dendritic spine analysis in animals infected with AAV-GFP or AAV-∆FosB after repeated cocaine. Data are presented as mean ± SEM.
firmed after behavioral testing (Fig. 2A). G9a overexpression significantly decreased the preference for cocaine in comparison to that seen in animals overexpressing GFP (Fig. 2B) and increased H3K9me2 levels in the NAc (Fig. 2C). Overexpression of a catalytically dead mutant of G9a (G9ah1093K) (14) did not affect cocaine preference (Fig. 2B) and had no effect on H3K9me2 levels in this brain region (Fig. 2C).

To further study the role of G9a in the behavioral effects of cocaine, and more specifically to mimic the repeated cocaine–induced repression of G9a expression in the NAc, adult G9a∆∆ mice (14) received intra-NAc injections of AAV vectors expressing Cre recombinase or GFP as a control. AAV-Cre knockdown of G9a in the NAc, which was confirmed immunohistochemically (fig. S5), significantly increased the effects of cocaine in place-conditioning experiments and decreased H3K9me2 levels in the NAc (Fig. 2, D and E). A commercially available pharmacological inhibitor of G9a and GLP, BIX02194 (15, 16), was used to ascertain whether enzyme inhibition similarly affects behavioral responses to cocaine. Indeed, pharmacological inhibition of G9a and GLP significantly increased the preference for cocaine and decreased H3K9me2 in the NAc (Fig. 2, F and G).

Repeated administration of cocaine increases the density of dendritic spines on NAc medium spiny neurons (17), a process associated with functional changes at excitatory glutamatergic synapses onto these neurons (18, 19) and sensitized behavioral responses to the drug (17, 20). We thus hypothesized that down-regulation of G9a activity in the NAc by repeated cocaine exposure might mediate cocaine’s ability to regulate the dendritic spine density of NAc neurons. Using ChIP with an antibody to G9a, we identified several putative G9a gene targets in the NAc, each of which has previously been implicated in cocaine-induced dendritic plasticity (Fig. 3A) (20–26). We found that repeated cocaine administration significantly decreased G9a binding, as well as levels of H3K9me2, at these gene promoters (Fig. 3B). In contrast, acute cocaine administration rapidly recruited G9a to some of these same gene promoters, which is consistent with increased G9a expression observed in the NAc 1 hour after an acute dose of cocaine (fig. S6). Although G9a binding at specific gene promoters correlates with changes in its expression, it remains unclear whether such events are mediated by altered global levels of G9a in the NAc and/or by differences in G9a recruitment after acute versus repeated cocaine administration.

Based on G9a’s regulation of numerous plasticity-related genes in the NAc, we directly examined whether maintenance of G9a expression in this brain region after repeated cocaine treatment was sufficient to block cocaine-induced dendritic spine formation. Using a cocaine treatment protocol previously demonstrated to promote dendritic spine induction in the NAc (20), we examined spine density in animals injected with either HSV-GFP or HSV-G9a. In agreement with previous findings, we observed a significant increase in dendritic spine density in the NAc after cocaine treatment, an effect that was blocked completely by G9a overexpression (Fig. 3C). G9a overexpression alone was not sufficient to decrease NAc dendritic spine density in the absence of cocaine. To complement these data, G9a∆∆ mice received intra-NAc injections of HSV-Cre, and spine density was quantified and compared to that in animals receiving HSV-GFP in the absence of cocaine. Knockdown of G9a expression significantly increased spine density on NAc medium spiny neurons (Fig. 3C).

Given the evidence that G9a down-regulation in the NAc after repeated cocaine treatment is mediated by ΔFosB, we next examined whether this transcription factor is likewise involved in the regulation of NAc dendritic spines. Although ΔFosB has not previously been linked causally to such dendritic plasticity, several of its targets, including Cdk5 and nuclear factor–κB subunits, have been so implicated (20–23); and ΔFosB’s persistent expression in NAc neurons correlates with increased dendritic spine density after repeated cocaine treatment (27). First, we found that induction of ΔFosB in bi-transgenic mice in the absence of cocaine, which down-regulated G9a and H3K9me2 expression (14), decreased G9a binding to the fosb gene (Fig. 3D, E). Conversely, overexpression of ΔFosB in the NAc of ΔJunD, a dominant negative mutant protein that antagonizes ΔFosB transcriptional activity, blocked the ability of repeated cocaine to increase dendritic spine formation in the NAc (Fig. 3C).

Our observation that ΔFosB regulates G9a expression in the NAc and that ΔFosB and G9a regulate some of the same target genes led us to examine other interactions between ΔFosB and G9a. After acute cocaine administration, when G9a levels were increased, binding of G9a to the fosb gene was increased, whereas after repeated cocaine administration, when G9a expression was suppressed, G9a binding to the fosb gene was decreased (Fig. 3A). Such decreased G9a binding after repeated cocaine was not observed for c-fos, where G9a binding is increased by repeated cocaine (fig. S7). This is consistent with the fact that, unlike fosb, c-fos is repressed, not induced, by chronic psychostimulant exposure (5). ΔFosB overexpression in bi-transgenic mice was sufficient to significantly decrease G9a binding to the fosb gene (Fig. 3D). Furthermore, G9a overexpression was sufficient to reduce increased ΔFosB expression after repeated cocaine administration (table S4). These data suggest an autorhythmic loop whereby G9a initially limits the induction of ΔFosB under acute cocaine administration. However, as ΔFosB accumulates with repeated drug exposure, it represses G9a and thereby potentiates its own further induction.

We have demonstrated that histone lysine methylation in the NAc is critically involved in regulating neuronal gene expression in response to cocaine. Repression of G9a and H3K9me2 after repeated cocaine administration promotes cocaine preference, in part through the transcriptional activation of numerous genes known to regulate aberrant forms of dendritic plasticity. Gaining a better understanding of the genes being regulated through such mechanisms will improve our knowledge of the complex biological basis of drug addiction and could aid in the development of more effective treatments for addictive disorders.

References and Notes
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Supporting Online Material
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