CellPress
REVIEWS

Special Issue: Biomarkers of Substance Abuse

Spotlight

HDAC5 Regulates the Formation of Drug Memories

Alexander C.W. Smith[1] and Paul J. Kenny[1,*]

Cocaine-associated environmental cues can precipitate craving and relapse in addicted individuals even after years of abstinence, but the molecular mechanisms by which maladaptive drug memories are generated remain unclear. New findings suggest that histone deacetylase 5 (HDAC5) plays a key role in this process.

Cocaine addiction has been described as a disorder of learning and memory in which environmental cues that generally accompany cocaine use, and as a consequence are repeatedly paired with the rewarding effects of the drug, gain motivational significance in their own right; these can trigger craving and relapse even after extended periods of abstinence [1]. Understanding the molecular mechanisms by which maladaptive associations are generated between cocaine and environmental cues is a major goal of addiction research. Similar to other forms of learning, changes in gene transcription are required for the formation and persistence of cue-triggered drug memories [2]. It is therefore not surprising that chromatin remodeling proteins such as HDACs and histone acetyltransferases, which inhibit and facilitate gene transcription, respectively, have been implicated in this process [3–5]. However, the precise mechanisms remain obscure. In a recent issue of *Neuron*, Taniguchi and colleagues [6] reported compelling new data suggesting that HDAC5 plays a key role in linking the rewarding effects of cocaine to cues in the environment, thereby modulating cue-triggered cocaine-seeking behavior.

HDAC5 is an activity-dependent HDAC that shuttles between the cytoplasm and the nucleus in a manner dependent on its phosphorylation status [4]. In the mammalian brain, depolarization of neurons can increase intracellular levels of cAMP, activating protein phosphatases that dephosphorylate HDAC5, causing its accumulation in the nucleus [5,7] (Figure 1). There, HDAC5 deacetylates histone proteins to inhibit the expression of target genes. Hence, HDAC5 can link changes in neuronal activity to alterations in gene expression required for the induction and maintenance of long-term neuronal plasticity [4]. Cocaine has been previously shown to activate Ca^{2+}/calmodulin-dependent protein kinase-II (CaMKII) to increase the concentrations of phosphorylated HDAC5 in the nucleus accumbens (NAc) of mice, a brain region that regulates the rewarding actions of cocaine [4], thereby enhancing its export from the nucleus and retention in the cytoplasm [5] (Figure 1). This, in turn, facilitates the expression of genes that can influence the rewarding actions of cocaine [4,8]. For instance, HDAC5/Hdac5 knockout (KO) mice demonstrate greater sensitivity to the rewarding effects of cocaine than wild-type control mice, as evidenced from conditioned place preference (CPP) procedures [4]. In these, increased time spent exploring an environment previously paired with cocaine injections relative to an environment paired with saline delivery, was considered as an index of cocaine reward in mice [4]. Conversely, virus-mediated expression in the NAc of a S279-phosphorylation deficient mutant HDAC5 (HDAC5-S279A; less efficiently exported from the nucleus), blunted the rewarding effects of cocaine in the CPP procedure [5]. Thus, the nuclear accumulation of HDAC5 is hypothesized to act as a molecular brake on cocaine reward that blocks the development of addiction, whereas its retention in the cytoplasm leads to the opposite effect, enabling addiction [5]. It is notable, however, that HDAC5-S279A blocked cocaine reward only when the mutant protein was delivered into the NAc in animals that had not yet undergone the conditioning sessions in the CPP procedure that allows cocaine injections to become associated with a specific environment in the CPP apparatus; by contrast, no effects were observed in animals that had already learned this cocaine–environment association [5]. This raises the intriguing possibility that HDAC5 might not be involved in the rewarding effects of cocaine *per se,* but instead, might regulate learned associations between cocaine and the environment.

To investigate this possibility, Taniguchi and colleagues recently developed a virus that expresses a triple mutant form of HDAC5 at S259, S279, and S498 residues (HDAC5-3SA), rendering the enzyme completely dephosphorylated, and sequestrated in the nucleus [6]. As expected, they found that expression of HDAC5-3SA in the NAc of mice attenuated the development of cocaine CPP [6]. Next, they investigated the effects of NAc expression of HDAC5-3SA in regulating intravenous cocaine self-administration behavior in rats. In this procedure, cocaine-seeking responses, such as lever presses, resulted in intravenous (IV) cocaine infusions and the simultaneous activation of discrete cues – such as the sensing of a cue light, located above the lever that delivered cocaine infusions [6]. These cues became strongly associated with delivery of the drug [6]. NAc HDAC5-3SA expression did not alter the number of cocaine infusions earned by the rats, suggesting that HDAC5 did not regulate the rewarding effects of cocaine that supported self-administration of the drug [6]. When cocaine

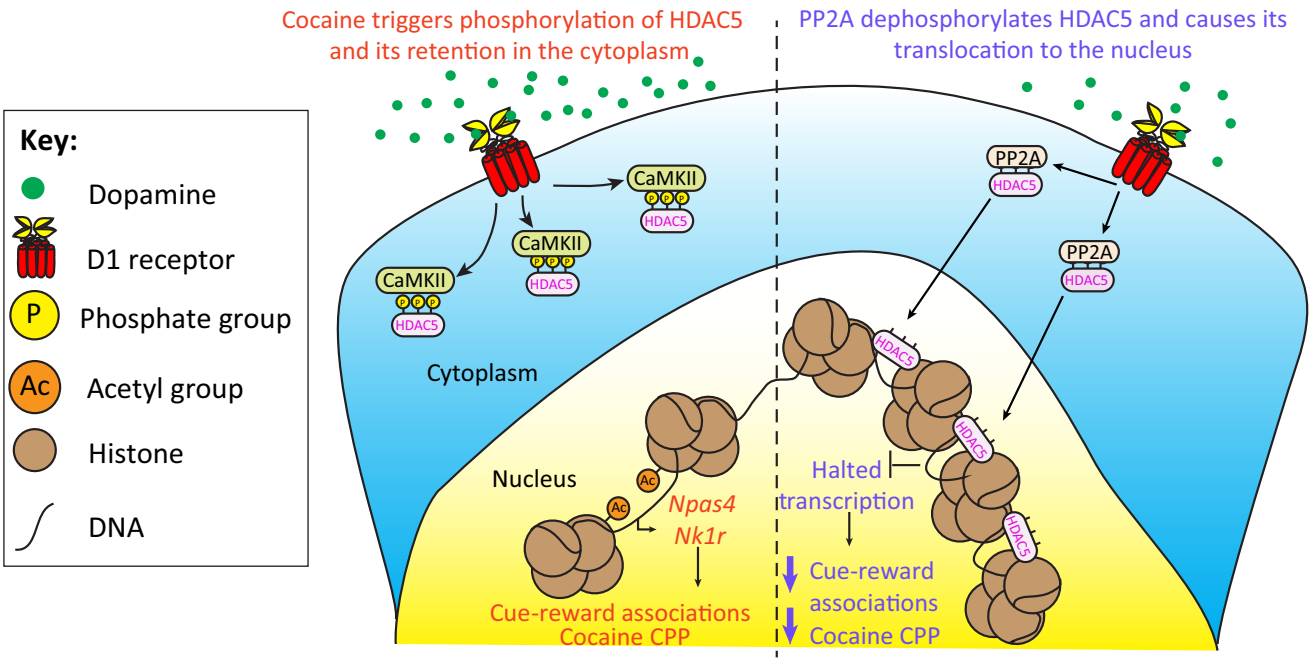

Figure 1. Effects of Cocaine on HDAC5 Phosphorylation and Target Gene Transcription in Rodents. Cocaine increases the levels of extracellular dopamine, which can activate CaMKII to increase the phosphorylation of HDAC5, resulting in its retention in the cytoplasm and enhanced transcription of cocaine-regulated genes. Conversely, HDAC5 is dephosphorylated by PP2A and translocated back into the nucleus, where it represses gene transcription. Abbreviations: CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; HDAC5, histone deacetylase 5; PP2A, protein phosphatase 2A.

infusions (and cocaine-paired cue lights) were withheld, control and HDAC5-3SA rats gradually decreased their cocaine-seeking responses across sessions at a similar rate [6]. However, when rats were exposed to cocaine-paired cue lights, as expected, there was a marked increase in cocaine-seeking responses in control rats, thought to reflect relapse-like cocaine seeking, although this effect was diminished in HDAC5-3SA rats [6]. This suggests that HDAC5 is not involved in cocaine reward; instead, it appears to regulate the mechanism by which environmental stimuli become associations with the actions of cocaine, and may be critical for the development of triggers that cause relapse during attempted abstinence.

Next, using chromatin immunoprecipitation coupled to next-generation sequencing, the study identified a large number of HDAC5 target genes [6]. One of these, *Npas4,* encodes an activity-regulated transcription factor involved in learning and memory processes and whose expression is increased by cocaine injections in the NAc [6]. RNAi-mediated knockdown of *Npas4* in the murine NAc blocked the rewarding effects of cocaine in the CPP procedure, similar to the effects observed with HDAC5-3SA expression [6]. Delivery of a Cre recombinase-expressing virus to the NAc of genetically engineered mice with LoxP sites flanking the *Npas4* gene, generated a conditional *Npas4* deletion in the mouse NAc (cKO^{NAc} mice), leading to a similarly decreased cocaine CPP response [6]. These findings suggest that HDAC5 acts in the NAc, at least in part, to control cocaine CPP by regulating *Npas4* expression.

Finally, the authors assessed IV self-administration behavior in *Npas4* cKO^{NAc} mice, noting that mutant mice self-administered similar amounts of cocaine as control mice did [6], consistent with the notion that HDAC5, and its target genes, are not involved in the rewarding effects of cocaine. However, KO mice took longer to learn how to reliably respond for the drug [6], suggesting that the animals exhibited reward-relevant learning deficits. In addition, cKO^{NAc} and control mice extinguished their cocaine-seeking responses when the drug and drug-paired conditioned stimuli were withheld; however, mutant mice again presented deficits in this behavior during early stages of this new learning experience, reinforcing the idea that this protein is involved in forming drug-relevant memories rather than regulating the rewarding effects of the drug [6]. Of note, cocaine-paired cues evoked cocaine-seeking responses to a similar degree in control and *Npas4* cKO^{NAc} mice [6]. This is surprising as it

suggests that Npas4 may not be involved in regulating cue–cocaine associations. How can this observation be reconciled with the marked impairment in CPP deficits seen in *Npas4* cKO^{NAc} mice described above? One possibility is that *Npas4* cKO^{NAc} mice simply learn cue–cocaine associations at a slower rate than control mice do, but this remains to be tested. In the CPP procedure used by the authors, mice had the opportunity to learn about cocaine-paired environmental cues during two conditioning sessions (i.e., they had only two opportunities to form new cocaine–cue associations) [6]. By contrast, in the cocaine self-administration procedure, mice had the opportunity to learn about cocaine-paired cues on each occasion that they earned an infusion. This translated into having many hundreds of occasions across the daily self-administration sessions to form cue–cocaine associations [6]. This being the case, it will be interesting to test whether the deficits in CPP observed in *Npas4* cKO^{NAc} mice might be ameliorated by conditioning the animals across a greater number of sessions. If so, this would suggest that HDAC5 control of *Npas4* expression in the NAc regulates the rate at which animals, and perhaps humans, form new associations between cocaine and environmental cues.

The findings by Cowan's group [6] revealed important new insights into the mechanisms of cocaine–cue associations, suggesting new avenues for future research. First, they unveiled the induction of the HDAC5-target gene *Npas4* in response to cocaine treatments – most prominently in the very sparse populations of rodent GABAergic interneurons in the NAc. Little is currently known about the role of these neurons in cocaine craving or relapse. Hence, exploring the involvement of these cells in cocaine–cue associations is likely to reveal new insights into mechanisms of relapse. Second, *Npas4* may contribute to deficits in cocaine–cue associations in mice where HDAC5 accumulates in the nucleus, but it is likely that other genes are also involved. Indeed, HDAC5 has been previously shown to regulate NAc expression of *Nk1r*, which encodes neurokinin 1 receptor, documented to inhibit cocaine reward in mice [4,8]. Hence, identifying other genes regulated by HDAC5 in the NAc is likely to be a fruitful avenue of future research. Third, it will be important to determine whether directly manipulating HDAC5 activity, or indirectly manipulating the activity or expression of HDAC5-targeted genes, may be a potential strategy to developing novel putative anti-relapse medications.

Acknowledgments

This work was supported by R01 DA025983 (PJK) and F32 DA043315 (ACWS).

[1]Department of Neuroscience, Icahn School of Medicine at Mount Sinai, One Gustave L. Levy Place, New York, NY 10029-6574, USA

*Correspondence: paul.kenny@mssm.edu (P.J. Kenny).

https://doi.org/10.1016/j.molmed.2017.12.011

References

1. Hyman, S.E. (2005) Addiction: a disease of learning and memory. *Am. J. Psychiatry* 162, 1414–1422
2. Renthal, W. and Nestler, E.J. (2008) Epigenetic mechanisms in drug addiction. *Trends Mol. Med.* 14, 341–350
3. Robison, A.J. and Nestler, E.J. (2011) Transcriptional and epigenetic mechanisms of addiction. *Nat. Rev. Neurosci.* 12, 623–637
4. Renthal, W. *et al.* (2007) Histone deacetylase 5 epigenetically controls behavioral adaptations to chronic emotional stimuli. *Neuron* 56, 517–529
5. Taniguchi, M. *et al.* (2012) Histone deacetylase 5 limits cocaine reward through cAMP-induced nuclear import. *Neuron* 73, 108–120
6. Taniguchi, M. *et al.* (2017) HDAC5 and its target gene, Npas4, function in the nucleus accumbens to regulate cocaine-conditioned behaviors. *Neuron* 96, 130–144.e6
7. Chawla, S. *et al.* (2003) Neuronal activity-dependent nucleocytoplasmic shuttling of HDAC4 and HDAC5. *J. Neurochem.* 85, 151–159
8. Kalivas, P.W. and Miller, J.S. (1984) Substance P modulation of dopamine in the nucleus accumbens. *Neurosci. Lett.* 48, 55–59