Neuropsychopharmacology

www.nature.com/npp

RESEARCH HIGHLIGHT

Gadd45b: at the intersection of transcriptional and epigenetic changes associated with cocaine-related behaviors

Mathieu E. Wimmer[1]

Neuropsychopharmacology (2021) 46:685–686; https://doi.org/10.1038/s41386-020-00887-2

The opioid epidemic has garnered much of the public's attention in recent years, but addiction to psychostimulants continues to represent a substantial proportion of the overall addiction crisis the US. Most addictive drugs increase dopamine release in nucleus accumbens [1]. In addition, repeated drug exposure causes long-lasting neural changes that remain in place long after the drug is no longer onboard. This enduring neural plasticity is thought to be mediated in part by epigenetic mechanisms [2], processes that modulate gene expression without altering the DNA sequence. Methylation of DNA at cytosine-phospho-guanine (CpG) dinucleotides can alter the expression of genes and contributes to neuronal changes that play a role in memory and behavioral effects of addictive drugs [3, 4]. A challenging and active research area is to delineate how psychostimulant-induced increased dopamine neurotransmission translates to transcriptional and epigenetic changes that cause long-lasting changes in addiction-related behaviors [5].

In a recent *Neuropsychopharmacology* publication, Zipperly et al. [6] address this important question using a comprehensive combination of in vivo and in vitro cutting-edge approaches. *Gadd45b*, a member of the Growth arrest and DNA-damage-inducible gene family is important for synaptic plasticity and memory formation. *Gadd45b* is also associated with decreased methylation at CpG islands within regulatory DNA regions [3, 4]. Hence, *Gadd45b* lies at the intersection of experience-dependent neuronal activity and downstream regulation of DNA methylation states [7, 8], making it a promising candidate to link dopamine neurotransmission to cocaine-induced plasticity and epigenetic changes.

Zipperly et al. [6] first showed that acute cocaine injections transiently elicits the transcription of *Gadd45b*, but not other enzymes involved in DNA methylation such as DNA methyl transferases or ten-eleven translocation enzymes in rat's nucleus accumbens. *Gadd45b* was the only component of the DNA methylation machinery tested that behaved like other immediate early genes such as *Arc*, *Fos* and *Egr1*—showing increased expression 1 h but not 24 h after acute cocaine injections. Moreover, cocaine sensitization or re-exposure to a cocaine-associated context was sufficient to increase nucleus accumbens *Gadd45b* expression, suggesting a potential role of this molecule in mediating cocaine's behavioral effects. Next, the authors directly tested the functional relevance of *Gadd45b* in the formation of cocaine-related memories. Constitutive *Gadd45b* knock out mice did not show cocaine conditioned place preference (CPP), suggesting that *Gadd45b* expression is necessary for cocaine-context associations. The authors then turned to a targeted gene knockdown approach using a CRISPR cas9 lentiviral construct designed to target exon 2 of *Gadd45b*. Knockdown of *Gadd45b* in the nucleus accumbens core of rats also prevented cocaine CPP, demonstrating that local *Gadd45b* is necessary for cocaine reward memory.

To further delineate the signaling pathways up- and downstream of *Gadd45b*, Zipperly et al. turned to a highly controllable rat primary striatal neuron culture system. Cultured neurons were first treated with dopamine (1 µM) for 1 h, a regimen that closely mimics the temporal dynamics of dopamine following cocaine administration in vivo. Dopamine increased expression of *Gadd45b* in striatal cells and this effect was blocked by co-treatment with the D1 dopamine receptor (Drd1) antagonist SCH-23390, suggesting that dopamine-induced expression of *Gadd45b* is mediated by Drd1 receptors. Consistent with this possibility, treatment with the Drd1 receptor agonist SKF-38393 also increased *Gadd45b* expression. In contrast, application of the D2/D3 dopamine receptor agonist quiporole did not change *Gadd45b* expression in cultured neurons. Drd1 receptors are coupled to the GαS G protein, which increases levels of cAMP intracellularly. The adenylyl cyclase activator Forskolin also increased *Gadd45b* mRNA expression in striatal neurons. Inhibition of the cAMP response element binding protein (CREB) and mitogen-activated protein kinase (MEK) signaling also blunted dopamine-elicited changes in *Gadd45b* mRNA expression. Together, these experiments demonstrate that dopamine-induced transcription of *Gadd45b* requires activation of Drd1 receptors and signaling through the cAMP, MAPK and CREB signaling pathways.

To further characterize the role of *Gadd45b* in regulating transcription and DNA methylation related to Drd1 activation, the authors designed a custom short-hairpin RNA (shRNA) tool to manipulate *Gadd45b* in cultured striatal neurons. RNA sequencing revealed that *Gadd45b* knockdown decreased expression of genes related to dopaminergic and glutamatergic synapses. To refine which gene programs were specifically engaged by Drd1 activation, the authors compared the transcriptome of neurons treated with either vehicle or SKF-38393 and expressing either control or *Gadd45b* shRNA. Interestingly, many of the genes that are most responsive to Drd1 activation in control neurons showed little response following SKF-38393 application when *Gadd45b* was knocked down, demonstrating that *Gadd45b* deletion prevents the full activation of dopamine-responsive gene programs in striatal neurons. The authors also examined whether DNA methylation patterns were affected by *Gadd45b* knockdown using reduced representation bisulfite sequencing. Surprisingly, only a small proportion of CpG sites were affected by *Gadd45b* knockdown, suggesting that this manipulation leaves baseline DNA methylation landscapes largely intact. In contrast, *Gadd45b* knockdown prevented the SKF-38393-mediated changes in

[1]Department of Psychology and Program in Neuroscience, Temple University, 1701 N 13th St, Philadelphia, PA 19122, USA
Correspondence: Mathieu E. Wimmer (Mathieu.wimmer@temple.edu)

Received: 16 September 2020 Accepted: 5 October 2020
Published online: 19 October 2020

methylation in striatal cells, suggesting that *Gadd45b* is critical for both hypermethylation and hypomethylation of loci following Drd1 receptor activation.

Lastly, the authors examined whether *Gadd45b* knockdown affected the physiological properties of striatal neurons using a high-throughput multi-electrode array system. This approach allows for both baseline measurements and assessments following application of SKF-38393. Given the baseline changes in genes related to glutamate and dopamine signaling, the authors unexpectedly found that neurons transduced with *Gadd45b* shRNA did not differ from scrambled controls in spontaneous activity, firing rate, mean action potential burst frequency, or number of spikes in each burst. Only burst duration was reduced by *Gadd45b* deletion. Moreover, SKF-38393 application increased action potential firing rate in both control and *Gadd45b* shRNA-expressing cells, suggesting that *Gadd45b* knockdown did not alter the electrophysiological changes associated with Drd1 receptor activation in striatal neurons.

Collectively, these findings highlight the importance of *Gadd45b* in the nucleus accumbens downstream of Drd1 activation in orchestrating transcriptional and epigenetic alterations that contribute to cocaine physiological and behavioral effects. A better understanding of the neural mechanisms associated with cocaine reward and dopamine signaling lays the groundwork to develop improved therapeutics for substance abuse. Treatments that go beyond treating symptoms associated with drug use and target the underlying long-term brain changes are needed. This elegant and comprehensive study contributes to these efforts and also addresses important aspects of reward-related memory formation. The authors' findings have broad implications to multiple research disciplines and raise some interesting questions about the molecular underpinnings of reward memories.

FUNDING AND DISCLOSURE
The author declares no competing interests.

ACKNOWLEDGEMENTS
I would like to thank Dr. Yavin Shaham for comments on the original version of this paper.

ADDITIONAL INFORMATION

REFERENCES

1. Di Chiara G, Imperato A. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA. 1988;85:5274–8.
2. Walker DM, Nestler EJ. Neuroepigenetics and addiction. Handb Clin Neurol. 2018;148:747–65.
3. Campbell RR, Wood MA. How the epigenome integrates information and reshapes the synapse. Nat Rev Neurosci. 2019;20:133–47.
4. Puckett RE, Lubin FD. Epigenetic mechanisms in experience-driven memory formation and behavior. Epigenomics. 2011;3:649–64.
5. Day JJ, et al. Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. Nat Neurosci. 2007;10:1020–8.
6. Zipperly ME, et al. Regulation of dopamine-dependent transcription and cocaine action by Gadd45b. Neuropsychopharmacology, 2020. https://doi.org/10.1038/s41386-020-00828-z.
7. Sultan FA, Sweatt JD. The role of the Gadd45 family in the nervous system: a focus on neurodevelopment, neuronal injury, and cognitive neuroepigenetics. Adv Exp Med Biol. 2013;793:81–119.
8. Ma DK, et al. Neuronal activity-induced Gadd45b promotes epigenetic DNA demethylation and adult neurogenesis. Science. 2009;323:1074–7.