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Epigenetics of Nicotine: Another Nail in the Coughing

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Abstract

In a mouse model, chronic nicotine exposure before cocaine use exacerbated the epigenetic, gene-expression, electrophysiological, and behavioral effects that occur during the transition from acute to chronic responses to cocaine that have been linked with the addictive process. Nicotine enhancement of the effects can be mimicked with an inhibitor of chromatin-modifying enzymes (class I and II histone deacetylases). These findings may spur the discovery of therapeutics for the treatment of addiction.

In case one needed more reasons for promoting stricter tobacco regulations, new research in this issue of *Science Translational Medicine* dissects the mechanism by which chronic nicotine exposure enhances the behavioral response of mice to cocaine (1). This rewiring of the rodent brain occurs through the inhibition of chromatin-modifying histone deacetylases and the resulting changes in gene expression. These previously unrecognized epigenetic effects of nicotine could lead to new targets for addiction medications.

The gateway theory of drug use is based on the frequently observed progression from tobacco or alcohol use to the use of marijuana and on to the abuse of other drugs (2). This model has been a dominant and influential force in the design of prevention strategies for substance-use disorders, despite frequent reports of violations to the rule and confounding factors (3-5).

Perhaps because of its commanding prevalence and illegal status, marijuana has attracted much attention as a possible major gateway drug for young people (6). Still, one must acknowledge the consistent evidence that shows that in most cases, tobacco use—and thus nicotine—precedes marijuana and that very early experimentation with cigarettes increases the likelihood of further experimentation with marijuana and then with other drugs (2). One wonders whether the prevailing focus on marijuana as a putative premier precursor drug might have kept researchers from more seriously exploring the possibility that nicotine—which is, in fact, one of the two drugs (the other being alcohol) that children and adolescents are most likely to obtain first—could have a stronger claim to that moniker.

Although the cumulative epidemiological data suggest this is the case, other approaches are needed to characterize the underlying mechanism(s) of nicotine priming. For example, a specific drug-use sequence might reflect a common genetic vulnerability for substance abuse in general that becomes expressed mostly thanks to the substances that young people can easily procure (that is, legal drugs and marijuana) (7). Alternatively, there may be drugs for which the pharmacological effects result in long-lasting changes in the brain's reward circuitry that enhance the sensitivity to other drugs, thus increasing the vulnerability for their abuse and dependence (8).

Competing interests: The author is the director of the U.S. National Institute on Drug Abuse, which administered a grant awarded to carry out some of the work discussed herein.

The new work by Levine *et al.* (1) addresses this question and offers new insights into the molecular mechanisms by which nicotine primes the brain to enhance the rewarding effects of cocaine. Reminiscent of pioneering research on the long-lasting molecular alterations that underlie memory formation, the authors used a mouse model, in which they could easily control the sequence of administered drugs, to investigate whether chronic preexposure of the mice to nicotine could induce similar persistent changes in brain reward circuitry that modified the animals' subsequent responses to cocaine administration.

Levine *et al.* found that mice that received a 7-day course of nicotine administration before challenge with cocaine exhibited a 78% increase in conditioned place preference (CPP) when compared with mice who had not been treated with nicotine before receiving cocaine. CPP measures the amount of time an animal spends in compartments that had been previously associated with a positive stimulus or reward (in this study, cocaine) and is a well-established measure of drug-induced conditioning.

Conditioning is central to the addiction process and involves changes in synaptic transmission akin to those that underlie learning and memory (9). The authors also showed that nicotine pretreatment caused a 95% increase in cocaine-induced locomotor sensitization when compared with that of animals that also received cocaine but had not been pretreated with nicotine. This response is a common observation in studies of repeated cocaine administration and is used as an experimental paradigm to study the neuroplastic changes linked to addiction (10). These observations were consistent with the 27% accentuation in the reduction in long-term potentiation (LTP) that was detected in the nucleus accumbens—a brain region in which drug-induced dopamine increases are associated with reward—when cocaine was administered to chronic nicotine pretreated mice relative to mice that were either not pretreated or pretreated with just a single dose of nicotine. LTP had been previously associated with neuroadaptations in glutamate receptor signaling, which modulate dopamine responses to repeated drug exposures and to conditioned drug cues (11). Last, at the cellular level the authors found that 7 days of nicotine pretreatment boosted the increase in expression of the *FosB* gene after cocaine treatment by 61% when compared with that of mice that received cocaine but were not pretreated with nicotine.

FosB encodes the transcriptional regulatory protein FosB and its truncated splice variant delta FosB. This finding is particularly telling in light of previous studies that show that up-regulation of *FosB* gene expression (and thus an increase in delta FosB) plays a pivotal role in driving the persistent neuroplastic changes that have been linked to drug addiction and other psychiatric conditions (12-15). Importantly, reversing the order of drug administration (pretreatment with cocaine before nicotine challenge) was ineffective, indicating a specific “priming” effect of nicotine exposure. In other words, chronic nicotine accelerated the behavioral, electrophysiological, and gene expression patterns seen in the transition from the acute to chronic responses to cocaine that are linked with the addictive process (Fig. 1).

Paralleling previous findings that the long-lasting cellular changes implicated in learning and memory involve epigenetic modifications of DNA that alter gene expression patterns, the addiction process has been shown to be mediated by epigenetic changes in various regions along the chromatin, including in the area of the *FosB* gene promoter. In fact, the delta FosB transcription factor may constitute a marker for the manifestation of addictive behaviors. The present study revealed that relative to no nicotine preexposure, repeated nicotine treatment increased the acetylation of histones—nuclear proteins that package DNA into chromatin—globally in the striatum (where the nucleus accumbens is located) and specifically in the *FosB* promoter. Nicotine priming stimulated the acetylation of histones H3 and H4, whereas cocaine alone stimulated acetylation of only histone H4.

Histone acetylation is an epigenetic mark in the chromatin that favors gene expression and is modulated by the balance between histone acetyltransferase (HAT) and histone deacetylase (HDAC) enzyme activities, which add and remove, respectively, acetyl groups from histones. Nicotine enhanced histone acetylation by inhibiting HDACs and thus deacetylation, which favors gene silencing. This nicotine-induced epigenetic modification may give rise to an open chromatin structure that primes *FosB* for enhanced transcriptional activation after chronic cocaine use, thus facilitating or promoting cocaine-use behaviors. The ability of chronic, but not acute, nicotine exposure to act as an HDAC inhibitor had not been reported previously and could have far-reaching translational implications not only for nicotine and other drug addictions but also for a host of smoking-related illnesses, including cancer.

Effects similar to those that were observed in mice exposed to chronic nicotine administration (including the enhancement of *FosB* up-regulation and reduction of LTP in the nucleus accumbens by cocaine treatment) were also seen in mice after treatment with the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA). Whether HDAC inhibition by nicotine reflects a direct or indirect action remains to be elucidated. However, the fact that a single dose of SAHA, a direct HDAC inhibitor, was sufficient to enhance cocaine's effects suggests that nicotine—the effects of which required 7 days of exposure to manifest—is either less potent or acts through unidentified intermediaries.

CLINICAL AND PUBLIC HEALTH IMPLICATIONS

The results of this study present multiple translational opportunities (Fig. 2). From the clinical perspective, an understanding of the nicotine-triggered events that result in the enhancement of cocaine's rewarding effects provides potential targets for the development of new medications. For example, would drugs that influence histone acetylation—as reported by the authors for low doses of theophylline, an activator of histone deacetylase—be beneficial in the treatment of cocaine addiction? Also, because most cocaine abusers also smoke cigarettes, it is reasonable to speculate that the concomitant use of tobacco products may serve to enhance the reinforcing effects of cocaine and, thus, interfere with treatment interventions that aim to achieve cocaine abstinence without also pursuing smoking cessation. On the other hand, the cocaine-enhancing properties of chronic nicotine should also raise concerns about the use of nicotine replacement therapy among cocaine abusers, which could lead to worse outcomes than the use of alternative medications or behavioral interventions for smoking cessation.

However, the translation potential of these findings may extend well beyond addiction and into other areas unrelated to substance-use disorders. For example, because HDAC inhibitors have been shown to enhance memory in animal models (16), the possible HDAC inhibitory activity identified in this study suggests that nicotine analogs could become promising targets in the development of medications capable of enhancing learning and memory. Similarly, such compounds may turn out to be therapeutically beneficial in the treatment of other conditions for which the etiology has been traced to an abnormal hypoacetylation state, such as in the case of Rubinstein-Taybi syndrome—a rare (~1 in 100,000) genetic disease characterized by broad thumbs and toes, short stature, distinctive facial features, and varying degrees of intellectual disability (17).

As elegant studies often do, this one also introduces many new questions that warrant further investigation. For example, future studies will need to (i) revisit the effects of chronic exposure to nicotine during adolescence, a period of dramatically greater vulnerability to drug experimentation and addiction; (ii) tackle the critical goal of determining the cellular targets through which nicotine inhibits HDAC, the class (or classes) of HDAC affected by

nicotine, and the overlaps between these actions and the epigenetically driven, distinct patterns of desensitized (that is, less responsive to an up-regulating stimulus) versus primed (more responsive to an up-regulating stimulus) genes proposed to result from chronic cocaine use (18); and (iii) assess whether nicotine, by inhibiting HDAC, also primes the brain for enhanced responsiveness to nondrug stimuli associated with long-lasting neuroplastic changes, such as posttraumatic stress disorder or the transition from acute to chronic pain.

As discussed by the authors, the findings also have public health implications, for they suggest that effective interventions to prevent smoking may also prevent abuse of cocaine and possibly other drugs. In addition, we should also entertain the notion that inasmuch as exposure to HDAC inhibitors during pregnancy is linked to malformation of the fetus (teratogenicity) (19), the finding that chronic nicotine inhibits HDAC could help explain the teratogenic effects of this agent among mothers who smoke during pregnancy (20) and raises concerns about the potential adverse effects of using nicotine replacement therapy as a smoking cessation strategy for expectant mothers (21). In addition, we could even begin to investigate the intriguing possibility that some of the smoking-related pathologies that result from abnormal inflammatory responses in the lung—which have been associated with increased acetylation of histone H4 (22) and decreased HDAC type 2 activity (23)—could also stem from nicotine's HDAC inhibitory action (Fig. 2).

This study achieves that rare level of translational power that successfully links information gleaned from rigorous epidemiological studies [that is, nicotine often precedes cocaine abuse in a pattern associated with worse outcomes (2)] with the molecular and cellular mechanisms (epigenetic modification and genetic reprogramming by nicotine in brain reward regions) that are likely responsible for the underlying behaviors that drive trends at the population level.

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Fig. 1. Molecular switches in cocaine response

Chronic nicotine exposure before cocaine use exacerbates the epigenetic (1, HDAC inhibition leading to enhanced chromatin relaxation), genetic (2, superinduction of *FosB* expression), electrophysiological (3, enhanced LTP attenuation), and behavioral (4, increased locomotor activity and CPP) effects seen during the transition from the acute to the chronic responses to cocaine that have been linked with the addictive process. In this schematic cartoon, the top trajectory represents the faster rate of conversion from acute and controlled to chronic and compulsive cocaine intake by animals that have been chronically preexposed (7 days) to nicotine. –, minus chronic nicotine exposure; +, plus chronic nicotine exposure (7 days).

Fig. 2. Translational bonanza

Repeated exposure to nicotine inhibits HDAC activity. Whether direct or indirect, this effect of nicotine causes epigenetic changes that increase gene expression. Here, Levine *et al.* show that nicotine treatment increased the expression of *FosB* in the nucleus accumbens. Because the substrates for such actions are ubiquitously expressed throughout the brain and body, it is reasonable to speculate that regional nicotine-driven genetic reprogramming could help explain not only the priming of nicotine to cocaine's rewarding responses reported by Levine *et al.* but also other known effects of nicotine in other areas in the brain [such as the hippocampus, amygdala, prefrontal cortex (PFC), and habenula] and in the rest of the body (such as the lungs, placenta, and fetus). Thus, the new findings open a wide range of research and translational opportunities.