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REVIEW ARTICLE

The Gateway Hypothesis of substance abuse: developmental, biological and societal perspectives

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ABSTRACT
The Gateway Hypothesis describes how tobacco or alcohol use precedes marijuana and other illicit drug use. We review the epidemiological data, explore the underlying molecular mechanisms in mice and discuss the societal implications of the hypothesis, including the use of e-cigarettes by young people.

Conclusion: Our mouse model identifies biological processes underlying the hypothesis, showing that nicotine is a gateway drug that exerts a priming effect on cocaine through increased global acetylation in the striatum.

INTRODUCTION
The Gateway Hypothesis describes a developmental process of involvement in drugs in the general population in which certain drugs serve as a gateway for the use of other substances in a specific temporal sequence. In this review, we discuss the hypothesis from three perspectives. First, we review epidemiological data that first delineated the sequence that is typical in Western societies (1). Second, we describe recent studies on the molecular mechanisms underlying the gateway effect (2). Finally, we consider briefly some of the implications of the gateway perspective for understanding broad trends in drug use in the population across different birth cohorts. As an epilogue, we discuss the implications of the findings as regards the use of e-cigarettes, especially by young people. A related review has previously been published (3).

The epidemiological underpinning of the Gateway Hypothesis
The prevalence of the use of different drug classes varies greatly. For example, in 2012, the lifetime prevalence of use in individuals aged 18–34 years in the United States ranged from 88.4% for alcohol, to 67.3% for cigarettes, 52.7% for marijuana, 16.4% for cocaine and 2.1% for heroin (4). The mean ages of onset were 15.6 years for cigarettes, 16.5 for alcohol and marijuana, 19.2 for cocaine and 20.1 for heroin. These ages of onset provide some insight into potential sequences of involvement into various drug classes. Most marijuana users (64.6%) started smoking or drinking before they started using marijuana, with 22.6% starting at the same age, 12.4% starting marijuana first and 4% starting smoking first. The patterns are even more striking for cocaine: 96.9% started smoking or drinking first, 2.2% started all three drugs at the same age, 0.8% started cocaine first, and 0.1% never smoked nor drank (Fig. 1).

Thus, in the general population in the United States and in other Western societies, there is a well-defined sequence of progression of drug usage. Drug usage starts with a legal drug and proceeds to illegal drugs. Typically, the use of tobacco or alcohol precedes the use of marijuana, which in

Key notes
- The Gateway Hypothesis describes the developmental sequence of drug involvement in which tobacco or alcohol use precedes the use of marijuana and other illicit drugs.
- We review the epidemiological data, explore the underlying molecular mechanisms and discuss the societal implications of the hypothesis, including young people's use of e-cigarettes.
- Our mouse model identifies biological processes underlying the hypothesis, showing that nicotine is a gateway drug that exerts a priming effect on cocaine through increased global acetylation in the striatum.

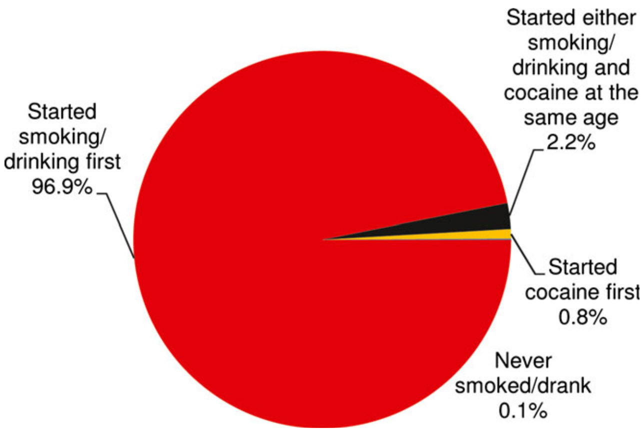

Figure 1 Most cocaine users have smoked cigarettes or drank alcohol before starting to use cocaine (U.S. population, ages 18–34, National Survey on Drug Use and Health 2012).

turn precedes the use of cocaine and other illicit substances. The main pathways and the minor ones are displayed in Figure 2. Even in the current period, when rates of marijuana use among youths have greatly increased and surpass those of cigarette use, most of those who have experimented with marijuana or cocaine first experimented with cigarettes or alcohol. Thus, in 2012, 56.3% of the high school seniors in the Monitoring the Future survey stated that they started smoking or drinking before using marijuana, with 30.7% saying that they started at the same age and 13% saying that they initiated marijuana use first. The comparable rates for cocaine were 84.5%, 14.1% and 1.4%. The existence of a regular developmental sequence of involvement in drugs of abuse is one of the most consistent findings in the epidemiology of drug use (1,5–9). Thus, in an analysis of representative samples of 17 countries, Degenhardt et al. (10) reported a very significant association between the use of alcohol and tobacco with cannabis use in all countries, except Japan, and between cannabis and other illicit drugs in all 17 countries. However, entry into a lower stage drug does not inexorably leads to a higher stage drug. Furthermore, the Gateway Hypothesis addresses developmental patterns of initiation of the use of different drugs. It does not directly address the development of dependence on these drugs.

An alternative to the Gateway Hypothesis has been proposed, based on the notion that the use of multiple drug classes reflects a common liability for drug use and that this liability, rather than the use of a particular drug, increases the risk of progressing to the use of another drug (11–13). Generalised risks include common genetic predispositions, psychosocial factors conducive to using drugs and environmental factors, including drug availability and opportunities for using drugs (6,13–20). However, even strong advocates of a Common Liability Model have found that the majority of their sample followed the gateway sequence. Thus, 77.6% of 124 marijuana users first used alcohol and, or, tobacco (21). Our position is that the Gateway Hypothesis and the Common Liability Model are complementary. Common factors explain the use of drugs in general, while specific factors explain why young people use specific drugs and do so in a particular sequence (8,22). In fact, population studies have found both generalised risk across substances and substance-specific risk, particularly risks attributable to tobacco use. As documented by Huizink et al. (8), smoking directly affects initiation to illicit drugs, although common genetic influences are sources of generalised risks across substances. This may be especially true in the case of drug dependence, as opposed to drug initiation, although specific risk factors for dependence on legal and illegal drugs have been identified (22).

Surprisingly, little progress has been made in addressing the two fundamental questions that derive from the observation that the use of one class of drug is followed by the use of another class. Firstly, does the use of the first drug cause the use of the second drug, and secondly, what mechanisms underlie the progression of drug use? Although epidemiological studies have established the sequence between substances and specified their association, epidemiological studies cannot establish a causal progression, nor can they identify the underlying cellular and molecular mechanisms that could contribute to the gateway sequence of drug use.

To test the causal validity of the Gateway Hypothesis, we undertook a programme that we had advocated earlier (23,24) to carry out research in an animal model, in which the investigator would administer one drug and observe

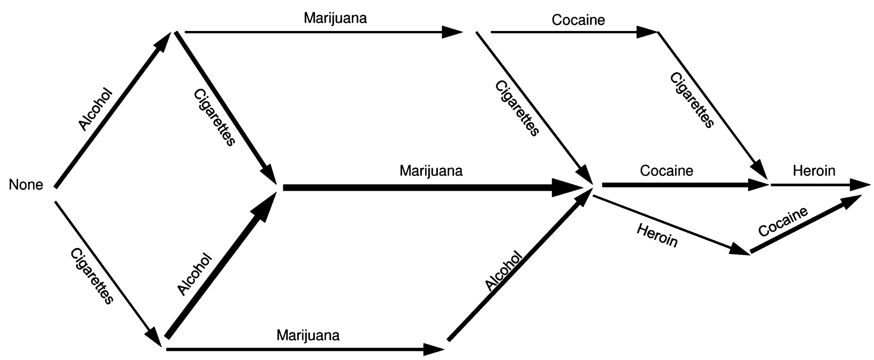

Figure 2 Pathways of drug involvement.

how it influences the reaction of the animal to a second drug. The investigator would then be able to change the order of drug exposures and observe the effects on outcomes.

From epidemiology to molecular biology: translational epidemiology

To obtain biological insights into the transition from nicotine to cocaine in the development of drug abuse, we bridged the epidemiology of drug use and molecular biology by developing a mouse model that explored the behaviour, physiology and molecular mechanisms underlying the gateway sequence.

Animal models can provide a rigorous test of drug use progression, in which drug-taking behaviour can be observed in relation to well-defined prior experiences with specific drugs, independently of any social or legal constraints regulating and defining drug use. Alternate specifications of the sequential order of drug presentation can help resolve the possibility that the ordered use between any two drugs is only determined by social factors related to the availability of different substances. In 2003, this paper's co-author Denise Kandel (23) wrote an editorial in the *Journal of the American Medical Association* advocating the use of animal models to address the Gateway Hypothesis. In collaboration with Amir Levine, Luca Colnaghi and Yan-You Huang, we developed a biological model system of the gateway sequence and applied it to mice (2). In mice, one can readily control the ordered use between two drugs so that order becomes the only experimental determinant of outcome, and other factors, such as the relative availability of different substances, are not relevant.

As a first step, we examined the sequence between nicotine and cocaine. The early work of Wikler (25) established that addiction is a form of learning. Subsequently, this paper's co-author Eric Kandel (26) and his colleagues worked out the molecular steps whereby the gene transcription factor cyclic AMP response-element-binding protein (CREB) switches on long-term memory. More recently, Hyman et al. (27) found in the striatum, a target in the brain that is critical for drugs of abuse, that the same molecular logic and the same molecular steps play a major role in addiction as those that have been found to underlie memory. These are activation of the transcriptional regulator of CREB, the key switch from short- to long-term memory and immediate early genes, such as FosB, and its isoform ΔFosB. Accumulation of ΔFosB is a crucial step in establishing addiction to most drugs of abuse and has been used as a molecular marker for these processes. Nestler found that knockout of FosB leads to decreased addictive behaviour (28). Levine et al. (29) and Nestler (30) found that cocaine leads to chromatin structure alteration in the FosB gene, due to histone acetylation of the promoter of the transcription factor ΔFosB.

If the gateway sequence is biologically causal, how does the causality work? Does use of a specific gateway drug, such as nicotine, enhance sensitivity to another drug, such as cocaine? Are the same molecular steps that have been found to play a role in addiction also involved? For example, is priming due to a remodelling of chromatin structure resulting from histone acetylation?

A TEST OF THE GATEWAY HYPOTHESIS

The sequential paradigm that we developed defined four experimental groups: nicotine followed by saline, nicotine followed by cocaine, cocaine followed by water and cocaine followed by nicotine. Water followed by saline was the control group. Nicotine was provided in the drinking water, and saline and cocaine were administered through intraperitoneal injection. We applied this paradigm at different levels of analysis: behavioural, neurophysiological and molecular.

Behaviour

In behavioural studies, we examined how sequential administration of nicotine and cocaine alters locomotor sensitisation and conditioned place preference. These are two addiction-related behaviours whose response to drugs of abuse depends in large part upon the striatum. We found that nicotine dramatically enhances the locomotor response to cocaine, by 98%, but cocaine does not alter the baseline activity of nicotine (Fig. 3). Mice pretreated with nicotine

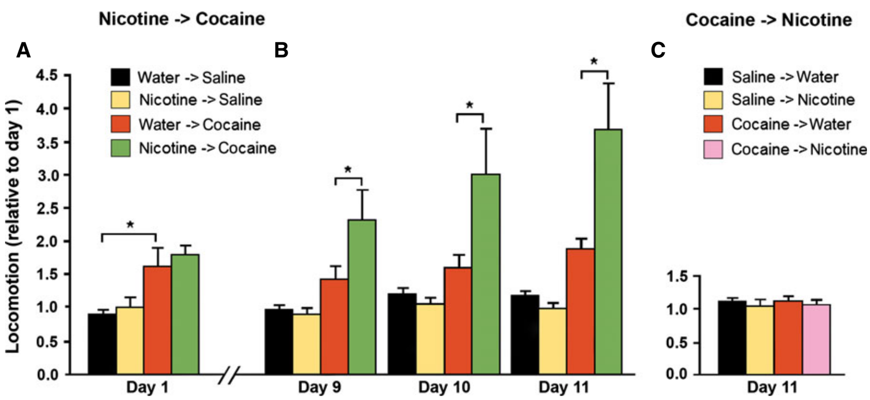

Figure 3 24-hour nicotine treatment has no effect on cocaine-induced locomotion (Panel A), whereas 7 days of nicotine treatment increases the locomotor effects of cocaine on days 9 through 11 (Panel B). By contrast, cocaine does not change the baseline activity of nicotine (Panel C). $^*p<0.05$.

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similarly showed a 78% further increase in place preference for the cocaine-coupled chamber compared with mice treated only with cocaine.

Cell and molecular biology

We then used the sequential drug administration paradigm to examine three cell biological and molecular markers of the priming effect of nicotine on cocaine and of cocaine on nicotine: synaptic plasticity, transcription of FosB and the recruitment of histone acetylation. We focused on the striatum, which is where most drugs of abuse exert their addictive effect.

In examining *synaptic plasticity,* we found that nicotine enhances the changes in long-term potentiation induced by cocaine, but cocaine has no effect on the action of nicotine on long-term potentiation (Fig. 4).

We next explored the *expression of FosB* and found that in the Nucleus Accumbens, the expression of FosB induced by cocaine is enhanced by pretreatment with nicotine, but FosB expression induced by nicotine is not enhanced with pretreatment with cocaine (Fig. 5).

Finally, we explored *histone acetylation and gene expression.* Chromatin is a combination of DNA and the protein spools (nucleosomes) around which the DNA is wrapped. The nucleosomes are made up of two copies of four different types of histone proteins, H1, H2, H3 and H4, around which the DNA winds. Thus, chromatin structure equals gene expression. The cascade of gene expression is initiated by the acetylation of histone tails by the CREB-binding protein (CBP). This neutralises the positively charged lysine residues on the histone tails that interact with DNA, thereby decreasing the affinity of histones for DNA, opening up the promoter and recruiting the machinery for transcription: the TATA box-binding protein and polymerase II. This, in turn, allows transcription of ΔFosB to occur. We found that cocaine acetylates the FosB promoter site only on histone H4, whereas nicotine acetylates both histones H3 and H4 and does so to a degree that cocaine cannot enhance it further (Fig. 6). Furthermore, although cocaine acetylates H4 as much as nicotine does, it only does so locally at the FosB promoter. In contrast, nicotine leads to more widespread acetylation of H3 and H4 throughout the whole striatum. Nicotine accomplishes this increase in widespread histone acetylation by inhibiting histone deacetylase, the enzyme that removes the acetylation (HDACs), thereby creating an environment primed for the induction of gene expression (FosB). As a result of the inhibition of HDAC, cocaine can now allow transcription to go on for a long time. The acetylated chromatin induced by nicotine exposure then allows greater FosB gene expression in response to cocaine injection than cocaine alone. This model is illustrated in Figure 7.

The priming effect of nicotine on cocaine-induced responses requires that nicotine be continuously administered when cocaine is given. Nicotine is administered for 7 days and needs to be administered concurrently on the eighth day when cocaine is introduced. There was no enhancement of the locomotor effect of cocaine (sensitisation), long-term potentiation or FosB expression after 14 days, if nicotine treatment was stopped before cocaine was administered.

FROM MOLECULAR BIOLOGY TO EPIDEMIOLOGY: TRANSLATIONAL EPIDEMIOLOGY

Animal models suggest predictions to be tested in human populations

We found that priming of the cocaine response by nicotine requires both more than 1 day of treatment and concurrent exposure to nicotine with the first cocaine exposure. This

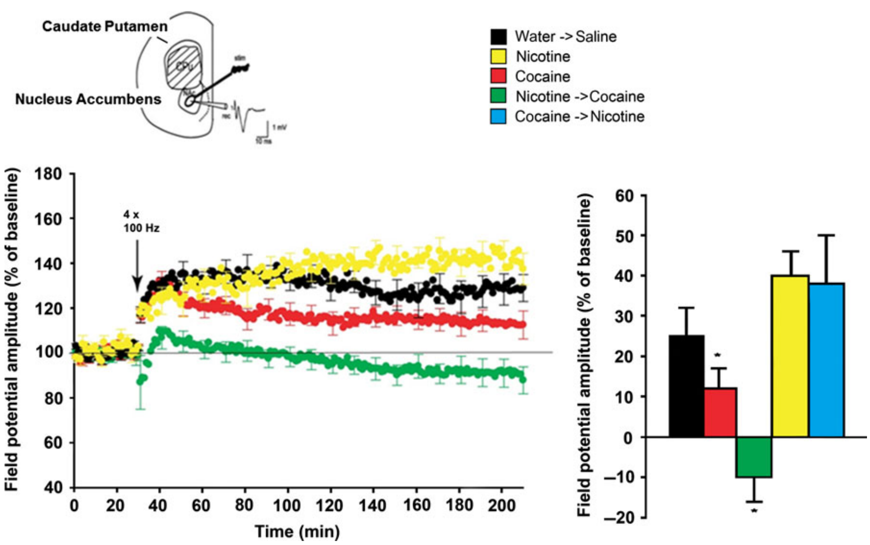

Figure 4 In the Nucleus Accumbens, nicotine enhances LTP changes induced by cocaine, but cocaine has no effect on the action of nicotine on LTP. HFS = High-frequency stimulation. *p<0.05

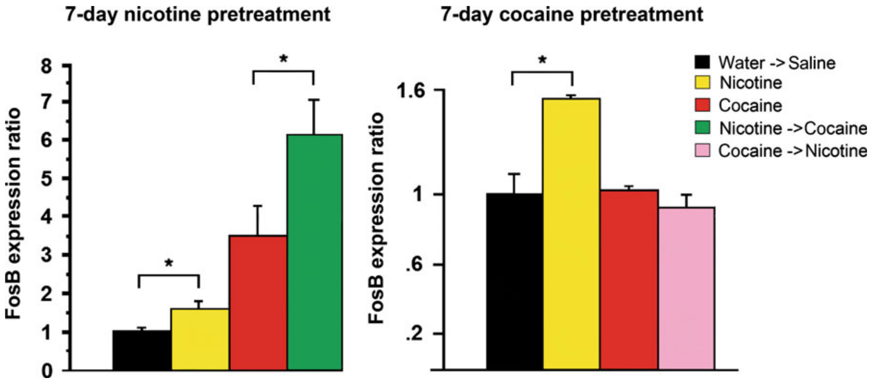

Figure 5 The expression of FosB induced by cocaine is enhanced by pretreatment with nicotine, but FosB expression induced by nicotine is not enhanced with pretreatment with cocaine. *p<0.05

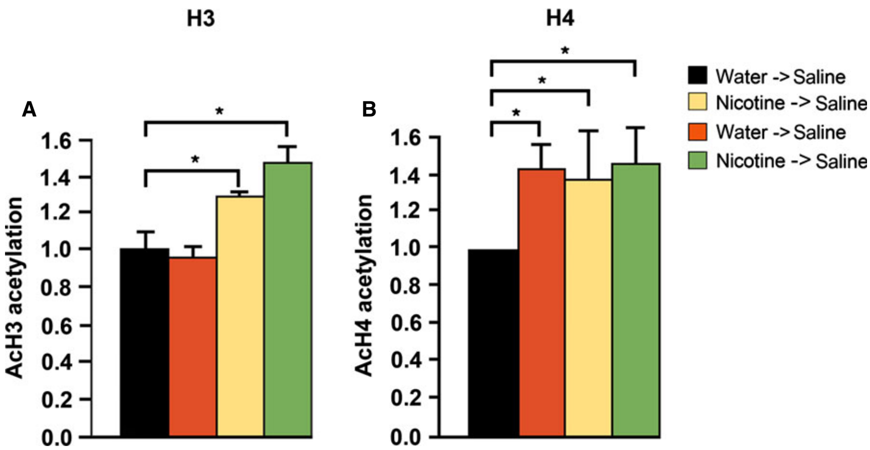

Figure 6 Nicotine, but not cocaine, induces histone hyperacetylation in the striatum at the FosB promoter. (A) histone H3 (K9); (B) H4 (K5 to K16). *p<0.05

finding prompted us to return to human populations and ask two questions. What is the smoking status of cocaine users at the time they start using cocaine? Does onset of cocaine usage, while actively smoking, result in enhanced effects of cocaine and higher rates of cocaine dependence? To address these questions, we re-examined existing data from a small longitudinal cohort of former high school students followed from the ages of 15.7–34.2 years (31). The majority of cocaine users (75.2%) were smoking during the month of cocaine onset. We also analysed data from the National Epidemiologic Study of Alcohol and Related Conditions (32), a large national cohort representative of the U.S. population. The rate of cocaine dependence was highest among cocaine users who initiated cocaine after having smoked cigarettes (20.2%) and was much lower among those who initiated cocaine before smoking (6.3%) or who had only smoked fewer than 100 cigarettes (10.2%).

SOCIETAL IMPLICATIONS

The Gateway Hypothesis has implications for understanding the behaviour of individuals at the societal level. We postulate that, at the population level, the drug behaviour of adults from different birth cohorts will be shaped by patterns of drug use they experienced as adolescents. As a consequence, in different historical periods, the levels of cocaine consumption in the adult population will vary as a function of the societal levels of smoking experienced by different cohorts in adolescence. To test this hypothesis, longitudinal data from adolescence to adulthood in different birth cohorts would be optimal. However, in the absence of these data, we used a suitable alternative to multiple longitudinal samples: repeated cross-sectional surveys from representative national samples, as the age groups in the surveys are a random subsample of their respective birth cohorts.

In collaboration with Kerry Keyes and Ava Hamilton from the School of Public Health at Columbia University, we started a limited test of the Gateway Hypothesis. We examined the drug behaviour of 12th graders (last year of high school in the United States) as a function of the levels of cigarette use they experienced 4 years earlier in the 8th grade, in 18 birth cohorts from 1991 to 2008. These cohorts spanned a period of lower smoking prevalence among 8th

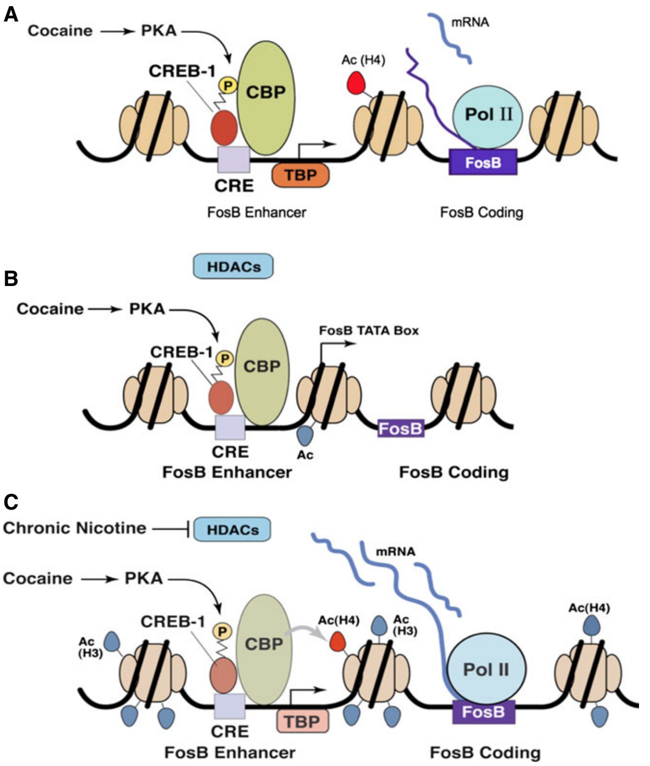

Figure 7 A molecular model for the nicotine–cocaine gateway sequence of drug usage. (A) FosB promoter region at baseline. (B) Acetylation of the promoter region of FosB after 7 days of nicotine exposure. (C) FosB expression in response to cocaine with previous nicotine exposure. TBP, TATA box-binding protein.

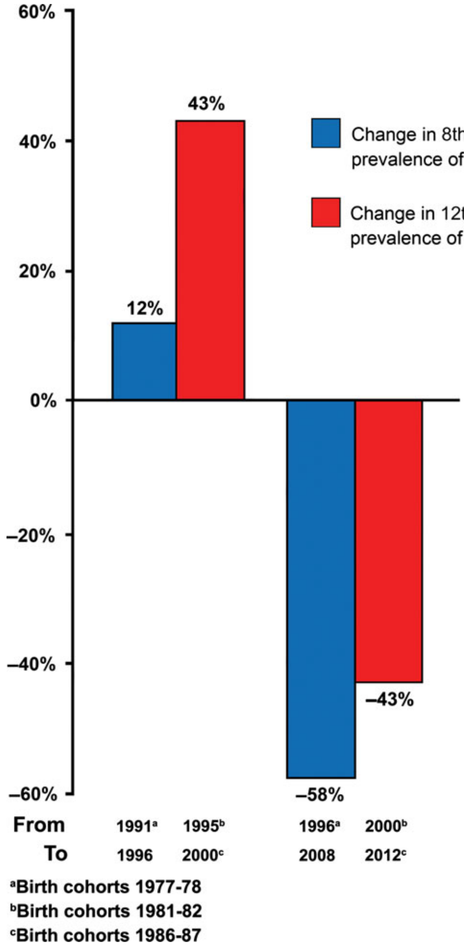

Figure 8 Proportional change in prevalence of smoking in 8th grade and cocaine use in 12th grade 4 years later among birth cohorts in periods when smoking prevalence increased and then decreased [Based on data from Monitoring the Future (42)].

graders (44% lifetime prevalence in 1991), a period of peak prevalence (49% in 1996) and a period of lower prevalence (20.5% in 2008). As predicted, periods of increase in lifetime smoking among 8th graders were followed by significant increases in rates of cocaine by seniors 4 years later. As lifetime smoking rates increased by 12%, lifetime cocaine rates increased by 43%. Correlatively, as smoking rates declined (by 59%), so did cocaine rates decline (by 43%) (Fig. 8).

DISCUSSION

Our results suggest a model (Fig. 7), whereby nicotine exerts its priming effect on cocaine through HDAC inhibition and provides a potential molecular explanation for the unidirectional sequence of drug use of nicotine on cocaine observed in mice and human populations. Thus, nicotine acts as a gateway drug and exerts a priming effect on cocaine in the sequence of drug use through increased global histone acetylation in the striatum, creating an environment primed for the induction of gene expression. Long-term synaptic plasticity in the Nucleus Accumbens is blocked when nicotine exposure is followed by cocaine treatment, which presumably relieves inhibitory constraints on dopaminergic neurons in the ventral tegmental area and leads to enhanced dopamine release when preceded by chronic nicotine exposure. For all measures – behavioural sensitisation, conditioned place preference, long-term synaptic potentiation and FosB gene expression – reversing the order of nicotine and cocaine administration is ineffective. Cocaine does not enhance the effect of nicotine. Importantly, the effect of nicotine on cocaine requires prior chronic, 7 day, exposure to nicotine. The priming effect does not occur when treating with nicotine for 24 h and then administering cocaine. Together, these results provide a biological basis and a molecular mechanism for the directionality of drug exposure observed in humans, whereby one drug affects the neural circuitry in a manner that potentiates the effects of another subsequently administered drug. Widespread acetylation of histones in the striatum by nicotine activates ΔFosB in response to cocaine, a gene related to reward and addiction, and thereby greatly enhances the rewarding effects of cocaine.

Moreover, we only observed the priming effect of nicotine when cocaine administration partially overlapped with nicotine exposure, suggesting that HDAC inhibition by nicotine depends on continuous nicotine intake. This is consistent with human epidemiological data, which similarly show that most individuals start using cocaine while concurrently using nicotine, a state that may enhance the physiological effects of cocaine. Concurrent smoking at onset of cocaine, in humans as in mice, is associated with greater persistence in cocaine consumption and addiction compared with not actively smoking when starting to use cocaine.

Finally, we documented at the population level how rates of smoking in adolescence impact on the prevalence of cocaine use in respective birth cohorts in early adulthood.

One can now ask whether the hyperacetylation produced by nicotine also a molecular explanation of drug action shared by the two other gateway drugs, alcohol and marijuana. Is there a single mechanism for all gateway sequences or does each sequence use a distinct mechanism? By having identified a specific biological mechanism that explains the sequence from cigarettes to cocaine in the population, we believe that the Gateway Hypothesis and Common Liability Model are complementary. Common factors will explain the use of drugs in general, while specific factors will explain why young people use specific drugs and do so in a particular sequence.

AND NOW TO E-CIGARETTES

The data that we present here can help inform the current debate about the potential benefits or harms of e-cigarettes, which have been presented as a tool for reducing smoking and the associated health burden in the population (33). Advocates emphasise its advantage as a clean product, without the morbidity associated with combustible tobacco. However, the users now include not only the chronic long-term smokers, the consumers that were originally envisaged, but adolescents and young adults, among whom use is increasing sharply. Thus, in the United States, the number of middle and high school students aged 11–17 using e-cigarettes doubled from 2011 to 2012 (34) and tripled from 2011 to 2013 (35). Discussions of the health effects of e-cigarettes focus on the lungs and the heart (36–38). None discuss the effect of e-cigarettes on the brain. The serious consequences on brain functioning of the kind we report here are not considered. The fact is that e-cigarettes are nicotine delivery devices that will have the same physiological effects on the brain, such as the acetylation effects that we have described, with attending addictive behaviours to a variety of drugs and experiences. Furthermore, the effects that we report are based on adult mice. We would expect these effects to be even stronger in adolescent animals. Indeed, pretreatment with nicotine has been reported to lead to enhanced cocaine-induced locomotor activity and increased initial self-administration of cocaine among adolescent but not adult rats (39,40). In addition, in rodents, nicotine treatment during adolescence enhances adult response to cocaine in adulthood (41). Whether e-cigarettes will prove to be a gateway to the use of combustible cigarettes and illicit drugs is uncertain, but it is clearly a possibility.

Rather than conveying the public health message that smoking is damaging to one's health, endorsement of e-cigarettes will implicitly condone smoking behaviour. In its descriptions and imagery, this is the message that is reinforced by the extensive advertising campaigns developed around the product. Its appeal to young people, reinforced by the availability of a multiplicity of flavours, during a critical period of brain development, presages a public health crisis.

Nicotine acts as a gateway drug on the brain, and this effect is likely to occur whether the exposure is from smoking tobacco, passive tobacco smoke or e-cigarettes. More effective prevention programmes need to be developed for all the products that contain nicotine, especially those targeting young people. Our data suggest that effective interventions would not only prevent smoking, and its negative health consequences, but also decrease the risk of progressing to illicit drug use and addiction.

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References

1. Kandel DB. Stages in adolescent involvement in drug use. *Science* 1975; 190: 912–4.
2. Levine AA, Huang YY, Drisaldi B, Griffin EA Jr, Pollak DD, Xu S, et al. Molecular mechanism for a Gateway Drug: epigenetic changes initiated by nicotine prime gene expression by cocaine. *Sci Transl Med* 2011; 3: 107ra109.
3. Kandel ER, Kandel DB. A molecular basis for nicotine as a Gateway Drug. *N Engl J Med* 2014; 371: 931–42.
4. Substance Abuse and Mental Health Services Administration (SAMHSA). *Results from the 2012 National Survey on Drug Use and Health: summary of national findings*. Rockville, MD: Substance Abuse and Mental Health Services Administration (SAMHSA), 2013.
5. Kandel DB, Yamaguchi K, Chen K. Stages of progression in drug involvement from adolescence to adulthood. *J Stud Alcohol* 1992; 53: 447–67.
6. Wagner FA, Anthony JC. Into the world of illegal drug use: exposure opportunity and other mechanisms linking the use of alcohol, tobacco, marijuana and cocaine. *Am J Epidemiol* 2002; 155: 918–25.
7. Kandel DB. *Stages and pathways of drug involvement: examining the Gateway Hypothesis, Cambridge*. UK: Cambridge University Press, 2002.
8. Huizink AC, Levälathi E, Korhonen T, Dick DM, Pulkkinen L, Rose RJ, et al. Tobacco, cannabis, and other illicit drug use among Finnish adolescent twins: causal relationship or correlated liabilities? *J Stud Alcohol Drugs* 2010; 71: 5–14.
9. Fergusson D, Boden J, Horwood LJ. Cannabis use and other illicit drug use: testing the cannabis gateway hypothesis. *Addiction* 2006; 101: 556–66.
10. Degenhardt L, Dierker L, Chiu WT, Medina-Mora ME, Neymark Y, Sampson N, et al. Evaluating the drug use "gateway" theory using cross-national data: consistency and

associations of the order of initiation of drug use among participants in the WHO World Mental Health surveys. *Drug Alcohol Depend* 2010; 108: 84–97.
11. Palmer RHC, Button TM, Rhee SH, Corley RP, Young SE, Stallings MC, et al. Genetic etiology of the common liability to drug dependence: evidence of common and specific mechanisms for DSM-IV dependence symptoms. *Drug Alcohol Depend* 2012; 123S: S24–32.
12. Vanyukov MM, Tarter RE, Kririlova GP, Kirisci L, Reynolds MD, Kreek MJ, et al. Common liability to addiction and “gateway hypothesis”: theoretical, empirical and evolutionary perspective. *Drug Alcohol Depend* 2012; 123(Suppl 1): S3–17.
13. Morral AR, McCaffrey DF, Paddock SM. Reassessing the marijuana gateway effect. *Addiction* 2002; 97: 1493–504.
14. Lynskey MT, Agrawal A, Henders A, Nelson EC, Madden PA, Martin NG. An Australian twin study of cannabis and other illicit drug use and misuse, and other psychopathology. *Twin Res Hum Genet* 2012; 15: 631–41.
15. Agrawal A, Neale MC, Prescott CA, Kendler KS. A twin study of early cannabis use and subsequent use and abuse/ dependence of other illicit drugs. *Psychol Med* 2004; 34: 1227–37.
16. Verweij KJ, Zietsch BP, Lynskey MT, Medland SE, Neale MC, Martin NG, et al. Genetic and environmental influences on cannabis use initiation and problematic use: a meta-analysis of twin studies. *Addiction* 2010; 105: 417–30.
17. Lessem JM, Hopfer CJ, Haberstick BC, Timberlake D, Ehringer MA, Smolen A, et al. Relationship between adolescent marijuana use and young adult illicit drug use. *Behav Genet* 2006; 36: 498–506.
18. Wilcox HC, Wagner FA, Anthony JC. Exposure opportunity as a mechanism linking youth marijuana use to hallucinogen use. *Drug Alcohol Depend* 2002; 66: 127–35.
19. Palmer RHC, Young SE, Hopfer CJ, Corley RP, Stallings MC, Crowley TJ, et al. Developmental epidemiology of drug use and abuse in adolescence and young adulthood: evidence of generalized risk. *Drug Alcohol Depend* 2009; 102: 78–87.
20. Cleveland HH, Wiebe RP. Understanding the association between adolescent marijuana use and later serious drug use: gateway effect or developmental trajectory. *Dev Psychopathol* 2008; 20: 615–32.
21. Tarter RE, Vanyukov M, Kirisci L, Reynolds M, Clark DB. Predictors of marijuana use in adolescents before and after licit drug use: examination of the Gateway Hypothesis. *Am J Psychiatry* 2006; 163: 2134–40.
22. Kendler KS, Myers J, Prescott CA. Specificity of genetic and environmental risk factors for symptoms of cannabis, cocaine, alcohol, caffeine, and nicotine dependence. *Arch Gen Psychiatry* 2007; 64: 1313–20.
23. Kandel D. Does marijuana use cause the use of other drugs? *JAMA* 2003; 289: 482–3.
24. Kandel D, Yamaguchi K. Testing the gateway hypothesis: an editorial. *Addiction* 2006; 101: 470–2.
25. Wikler A. On the nature of addiction and habituation. *Br J Addict Alcohol Other Drugs* 1961; 57: 73–9.
26. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. *Science* 2001; 294: 1030–8.
27. Hyman SE, Malenka RC, Nestler EJ. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci* 2006; 29: 565–98.
28. Hiroi N, Brown J, Haile CN, Ye H, Greenberg ME, Nestler EJ. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine’s psychomotor and rewarding effects. *Proc Natl Acad Sci USA* 1997; 94: 10397–402.
29. Levine AA, Guan Z, Barco A, Xu S, Kandel ER, Schwartz JH. CREB-binding protein controls response to cocaine by acetylating histones at the fosB promoter in the mouse striatum. *PNAS* 2005; 102: 19186–91.
30. Alibhai IN, Gree TA, Potashkin JA, Nestler EJ. Regulation of fosB and ΔfosB mRNA expression: in vivo and in vitro studies. *Brain Res* 2007; 1143: 22–33.
31. Kandel DB, Logan JA. Patterns of drug use from adolescence to young adulthood - I. Periods of risk for initiation, continued use, and discontinuation. *Am J Public Health* 1984; 74: 660–6.
32. Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA. Nicotine dependence and psychiatric disorders in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch Gen Psychiatry* 2004; 61: 1107–15.
33. Grana R, Benowitz N, Glantz S. E-cigarettes: a scientific review. *Circulation* 2014; 129: 1972–86.
34. Frieden T. *E-cigarette use more than doubles among U.S. middle and high school students from 2011–2012*. Centers for Disease Control (CDC), editor, 2013. Available from URL http://www.cdc.gov/media.releases/2013/p0905-ecigarette-use.html.
35. Bunnell R, Agaku I, Apelberg B, Caraballo R, King B, Arrazola R, et al. Intentions to smoke cigarettes among never-smoking US middle and high school electronic cigarette users, National Youth Tobacco Survey, 2011-2013. *Nicotine Tob Res* 2014; doi:10.1093/ntr/ntu166. In press: 23 pgs.
36. Bhatnagar A, Whitsel LP, Ribisl KM, Bullen C, Chaloupka F, Piano MR, et al. Electronic cigarettes: a policy statement from the American Heart Association. *Circulation* 2014; 130: 1418–36.
37. World Health Organization. Electronic nicotine delivery systems, in Conference of the Parties to the WHO Framework Convention on Tobacco Control; 2014, July 21; WHO Framework Convention on Tobacco Control; 13 pgs. Available from URL http://apps.who.int/gb/fctc/PDF/cop6/FCTC_COP6_10-en.pdf?ua=1.
38. Grana R, Benowitz N, Glantz S. Background paper on e-cigarettes, in Tobacco Control Paper Series; 2013, December; World Health Organization (WHO): University of California, San Francisco.
39. McQuown SC, Belluzzi JD, Leslie FM. Low dose nicotine treatment during early adolescence increases subsequent cocaine reward. *Neurotoxicol Teratol* 2007; 29: 66–73.
40. McQuown SC, Dao JM, Belluzzi JD, Leslie FM. Age-dependent effects of low-dose nicotine treatment on cocaine-induced behavioral plasticity in rats. *Psychopharmacology* 2009; 207: 143–52.
41. Kelley BM, Rowan JD. Long-term, low-level adolescent nicotine exposure produces dose-dependent changes in cocaine sensitivity and reward in adult mice. *Int J Devl Neurosci* 2004; 22: 339–48.
42. Johnston LD, O’Malley PM, Miech RA, Bachman JG, Schulenberg JE. *Monitoring the Future national results on adolescent drug use: overview of key findings*, 2013; Ann Arbor, MI: Institute for Social Research, the University of Michigan, 2014.