Research Findings
Vol. 22, No. 3 (April 2009)

Cocaine Locks Rats Into Unrewarding Behaviors

Brain circuits that guide behavior by registering consequences become less flexible after drug exposure.

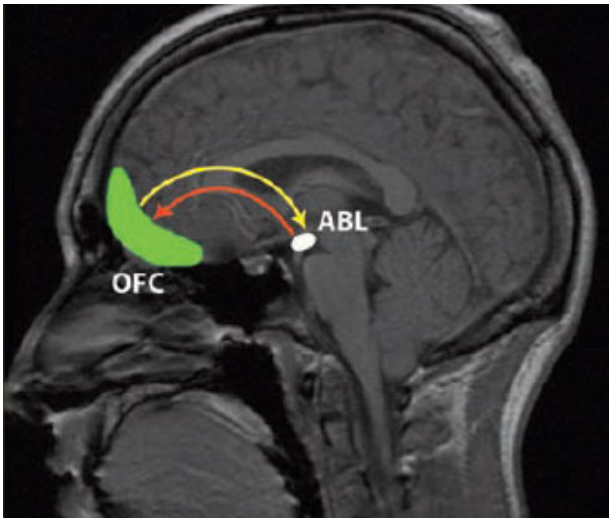
By *NIDA Notes* Staff

People initially take cocaine for pleasure, but for most chronic abusers, the high becomes progressively shorter and weaker, and negative social and economic consequences grow increasingly dire. Relationships hit the rocks, financial problems mount, and legal trouble follows, but the cocaine abuser often fails to adapt his or her behavior to avoid the accumulating personal disasters and instead remains stuck in self-damaging patterns.

New NIDA-funded research with rats indicates that cocaine may contribute to this inflexibility by impeding abusers' ability to associate warning signs with outcomes. The research links successful sign reading to two connected brain structures—the orbitofrontal cortex (OFC), located directly above the eye sockets, and the basolateral amygdala (ABL), deep in the brain. Cocaine appears to weaken neural signaling in these structures.

"Our findings may explain why cocaine abusers and cocaine-exposed animals have difficulty adapting their behavior to avoid negative outcomes," says Dr. Geoffrey Schoenbaum, who led the University of Maryland School of Medicine studies. "Cocaine seems to disrupt the information-processing ability of neurons in a learning circuit that helps animals and people accommodate their behavior when the environment changes."

CRITICAL INFORMATION PROCESSING
Research indicates that cocaine weakens neural signaling in a learning circuit between the orbitofrontal cortex (OFC) and the basolateral amygdala (ABL).



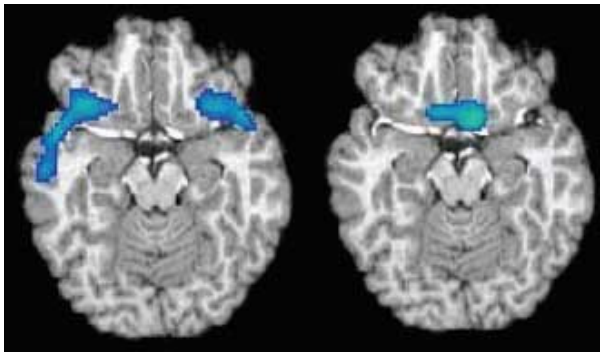
LEARNING TO USE CUES

To test cocaine's impact on learning and adaptation, Dr. Schoenbaum and colleagues used a protocol called the two-odor go/no-go discrimination task (see box). The protocol consists of two parts. The first tests an animal's ability to link cues to desired and aversive outcomes. It challenges the animal to perform a task analogous to that

of a person learning that right-hand faucets deliver cold water and left-hand ones, hot. In Dr. Schoenbaum's protocol, the counterparts to the faucets are odors. The researchers give a rat a whiff of one odor immediately before filling a well in the cage with a delectable sucrose-flavored drink, and they provide another odor when the well is about to be filled with a repugnant, quinine-flavored concoction. The rats have to learn to use both cues to obtain the sweet drink and shun the nasty one.

Brain Activity Differs in Cocaine Abusers According to Gender

Cocaine abusers have reduced neural activity in the orbitofrontal cortex (OFC), a brain region that mediates decisionmaking. NIDA-funded researchers have discovered that gender determines where in the OFC the dampening occurs.



LOCAL LULLS Abstinent cocaine abusers show gender-specific reduction of blood flow (blue) in the OFC. Below, differences between a male brain (left) and a female brain (right).

Dr. Bryon Adinoff and colleagues at the University of Texas Southwestern Medical Center and the Veterans Affairs North Texas Health Care System measured OFC neural activity, as indicated by blood flow, of 35 people who had used cocaine for 12 years, on average, but had been abstinent for 2 to 4 weeks. They compared the results with measurements from 37 people who had never used the drug. The researchers found that the OFC contributed a smaller portion of total brain activity in cocaine abusers than in nonabusers. However, the relative deficit was in the lateral OFC in men and in the medial OFC in women.

"One can hypothesize that sex differences in regional blood flow may give rise to contrasting behavioral responses," says Dr. Adinoff. Such differences might arise because the areas most affected in each gender support different behaviors. For example, brain scans of people who do not use drugs have suggested that the lateral OFC is active when people refrain from doing something that they anticipate will have a bad outcome. In contrast, the medial OFC engages when people take action to try to achieve a desired result.

The depressed neural activity in the lateral OFC among men who abuse cocaine may lead to problems putting the brakes on behaviors with bad outcomes and so hinder their ability to abstain, says Dr. Adinoff. The less active medial area in women may reflect a blunted drug reward, he adds.

While his findings are likely to be relevant for individuals in early abstinence, Dr. Adinoff notes that they may not apply to individuals in later stages of recovery. "The participants in our study had only been abstinent 2 to 4 weeks," he says. "Scientists need to examine whether the depressed neural activity we observed among cocaine abusers recovers with long-term abstinence."

"Future research might examine whether regional differences influence

treatment strategies and recovery success," notes Dr. Harold Gordon of NIDA's Division of Clinical Neuroscience and Behavioral Research.

Dr. Adinoff concurs, suggesting that through understanding these differences, treatment providers may eventually be able to tailor gender-specific therapies that promote abstinence.

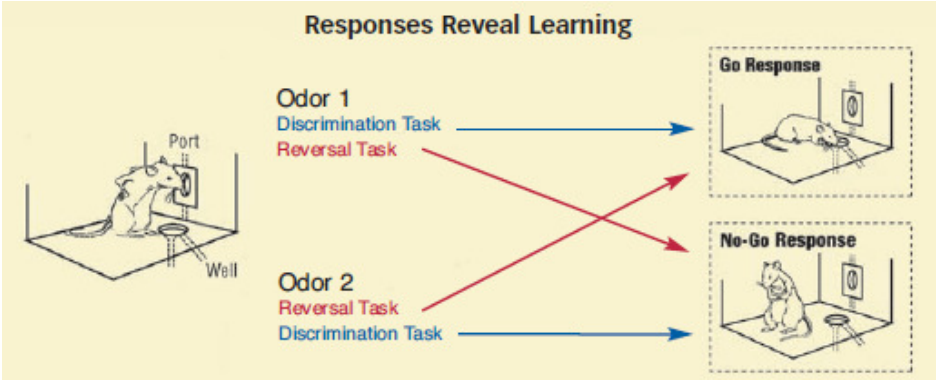
Source: Adinoff, B., et al. Sex differences in medial and lateral orbitofrontal cortex hypoperfusion in cocaine-dependent men and women. *Gender Medicine* 3(3):206-222, 2006. [Abstract]

The second part of the go/no-go protocol tests rats' ability to adjust when cues change their meanings. The odor that formerly indicated the sweet drink now signals the bitter one, and vice versa. This put the animals into a situation analogous to that of a person whose inattentive plumber crossed the pipes leading to a sink's faucets. A person in this predicament must quickly learn to change expectations or risk repeated scaldings.

Dr. Schoenbaum's team ran two groups of rats through the go/no-go protocol. One group had been exposed to cocaine daily for 2 weeks one month prior to the protocol, and the other was drug-free. In the first part of the protocol, both groups readily learned to discriminate between the odor cues. After a dozen trials, both groups consistently—though not unerringly—went to the well following the cue for sweet and shunned it following the cue for bitter.

TASKS TEST MENTAL FLEXIBILITY

Task 1—Go/no-go discrimination	**Task 2—Reversal**
Illuminated lights indicate an odor will be forthcoming when the rat pokes its nose in the port. The odor signals which liquid, either sucrose or quinine, will appear in the well after 3 seconds. Odor 1 predicts sucrose; odor 2 predicts quinine. The rat repeatedly experiences the association between each odor cue and its taste outcome.	Everything is the same as before, with a critical exception: The odors predict the opposite outcomes. Now, odor 1 predicts quinine; odor 2 predicts sucrose. The rat again repeatedly experiences the association between each odor cue and its taste outcome.
Interpreting the Response: As rats learn to discriminate between the odors and use each odor's predictive significance to obtain desirable taste outcomes, they will begin to consistently head for the well when the port contains odor 1 and avoid it when the port contains odor 2.	*Interpreting the Response:* As rats learn to reverse their expectations in line with the switched predictive significance of the two odors, they will increasingly head for the well when the port contains odor 2 and avoid it when the port contains odor 1.



One observation during the first part of the protocol suggested that, despite their

similar learning curves, the cocaine-exposed rats had reduced sensitivity to cues predicting negative experiences. The behavior of the rats in the two groups differed in those occasional instances where rats mistakenly went to the well following the cue for the bitter drink. The drug-naïve animals hesitated before setting off, suggesting that they had some inkling that the consequences might not be desirable. The drug-exposed animals, in contrast, rushed right to the well.

In the second part of the protocol, cocaine markedly reduced some rats' ability to adapt to the switched odor-drink pairings. The drug-naïve rats and half of the drug-exposed rats learned to reverse their responses to the cues after an average of 28 trials. The other half of the drug-exposed rats, however, required 35 trials.

NEURON FLEXIBILITY

The cocaine-exposed rats' poorer performance in the go/no-go protocols suggested that the drug impairs neurons in a brain circuit that links cues to the expectation of satisfaction or dissatisfaction. When a person or an animal responds to a cue—whether it be the position of a faucet or an odor—these neurons encode whether the experience that follows feels good or bad. In subsequent encounters with the cue, some neurons increase their firing rate if past responses led to a satisfying experience; others increase their firing rate if past responses caused aversive or disappointing outcomes.

"The firing of these neurons represents the linking of the cue to an expectation of an outcome, based on previous experience," says Dr. Schoenbaum. "We believe that at the time an animal has to decide whether or how to respond, these expectations influence its decision."

To test the hypothesis that cocaine exposure affects these outcome-expectant neurons, the research teams ran rats through go/no-go protocols while monitoring the animals' neuronal activity in two brain areas: the OFC and ABL. The OFC is part of the brain's decisionmaking circuit; its neuronal activity has been associated with stimulant addiction and craving. The ABL is part of the brain's emotional circuit. In previous studies, people with a damaged OFC or ABL were slow to change response patterns after consequences had changed from rewarding to adverse. This behavior resembles that of chronic cocaine abusers—and also some of the cocaine-exposed rats in the team's earlier experiment.

In both cocaine-exposed and unexposed rats, electrode recordings taken during the first part of the protocol showed that about 19 percent of the neurons monitored in the OFC and 26 percent of those in the ABL developed outcome-expectant firing patterns. This finding is consistent with the observation that the two groups of animals learned equally well to use the initial cues to guide their drinking.

Nevertheless, the two groups' neuronal responses may help explain why, on those occasions when the rats mistakenly responded to the quinine cue, the cocaine-exposed rats went directly to the well while the unexposed rats hesitated. The recordings revealed that the exposed rats' OFC quinine-predicting neurons failed to activate in response to the odor.

COCAINE EXPOSURE REDUCED THE ABILITY OF ABL NEURONS TO CHANGE RESPONSE DURING A REVERSAL TASK Researchers recorded the effect on individual ABL neurons during the two-odor go/no-go discrimination and reversal tasks.

	Changed Response	Failed to Change Response	Became Nonselective
Cocaine-exposed Rats	15%	27%	58%
Unexposed Rats	48%	3%	48%

In the second part of the go/no-go protocol, the cocaine-exposed animals' slower adaptation correlated with reduced flexibility of outcome-expectant neurons. When the researchers reversed the odor cues, neurons predicting sweet and bitter must switch their responses to continue to support decisions leading to happy drinking experiences.

Yet approximately 27 percent of those neurons in the ABL of the drug-exposed rats failed to make the switch—compared with only approximately 3 percent in the drug-free rats. The ABL neurons of the drug-exposed rats that initially signaled favorable expectations proved more inflexible than those that signaled unfavorable expectations.

A MODEL FOR DECISIONMAKING

Dr. Schoenbaum's results led him to propose a model to explain how the ABL and OFC interact in cue response decisions. In this schema, when an individual encounters a familiar cue, ABL outcome-expectant neurons send the OFC a "good/go" or "bad/don't-go" message, or no message at all. The OFC combines this message with information arriving from other brain areas to form a comprehensive picture of the likely consequences of acting.

This picture becomes the basis for a decision to respond to the cue or refrain. If the individual does respond, the OFC then compares the resulting consequences with the picture and notifies the ABL whether the latest data confirm or contradict the expectation. Completing the cycle, the ABL outcome-expectant neurons use this feedback to adjust their future responses to the cue.

"By weakening the responses of these frontal cortex areas, chronic cocaine use may make people more prone to relapse and compulsive drug-seeking."
—Dr. Geoffrey Schoenbaum

The key to cocaine abusers' persistent self-defeating behaviors is the drug's interference with the last step in this cycle, Dr. Schoenbaum says. Feedback from the OFC is weakened by drug exposure; consequently, ABL outcome-expectant neurons fail to change their responses. Instead, they persist in established firing patterns, continuing to signal outdated information to the OFC, and become a hindrance rather than a help to good decisionmaking.

"Cocaine renders the OFC and other frontal cortex areas' messages about likely outcomes less effective. Such signals both guide behavior and facilitate learning when things don't go as expected. By weakening the responses of these frontal cortex areas, chronic cocaine use may make people more prone to relapse and compulsive drug-seeking," says Dr. Schoenbaum.

"A person in drug abuse treatment is trying to change his or her behavior, yet these animal findings suggest that cocaine exposure ossifies a neural circuit likely involved in these changes," says Dr. Susan Volman of NIDA's Division of Basic Neuroscience and Behavioral Research. "Scientists may someday develop medications that enhance neural flexibility and facilitate reengagement of cognitive circuits, which would help behavioral therapy lessons sink in."

Dr. Elliot Stein of NIDA's Intramural Research Program, who is performing brain imaging of cocaine abusers as they do reversal learning tasks, agrees: "If the drug-exposed brain lacks plasticity for new learning, then restoring the functional integrity of the circuit may increase the effectiveness of behavioral interventions."

SOURCES

Stalnaker, T.A., et al. Abnormal associative encoding in orbitofrontal neurons in

cocaine-experienced rats during decision-making. *European Journal of Neuroscience* 24(9):2643-2653, 2006. [Abstract]

Stalnaker, T.A., et al. Cocaine-induced decision-making deficits are mediated by miscoding in basolateral amygdala. *Nature Neuroscience* 10(8):949-951, 2007. [Abstract]

Volume 22, Number 3 (April 2009)

Research Findings
Vol. 21, No. 4 (October 2007)

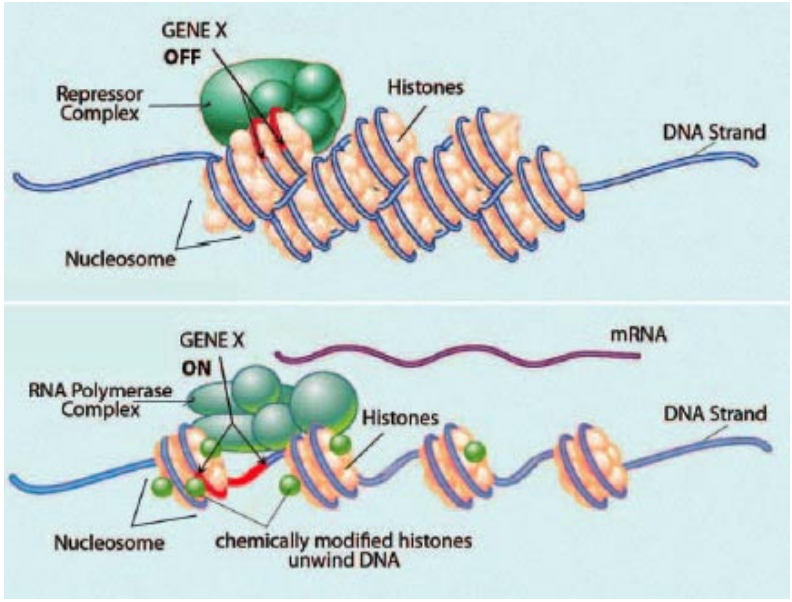
Gene Experiment Confirms a Suspected Cocaine Action

Building on knowledge from developmental and cancer biology, addiction researchers are learning how acute and chronic cocaine exposure regulates certain genes.

BY LORI WHITTEN, *NIDA Notes* Staff Writer

Cocaine produces the long-term brain changes that underlie addiction in part by activating certain genes. Dr. Eric Nestler and colleagues at the University of Texas Southwestern Medical Center and Harvard Medical School have shown that the drug achieves this activation at least in part through a process called chromatin remodeling.

CHROMATIN AND DNA HELIX FORM CHROMOSOMES
Nucleosomes slide apart during chromatin remodeling, increasing transcription factors' access to a gene and thereby activating it. The DNA will then make mRNA, the blueprint for protein production.



The finding opens up a new avenue for potential therapies for addiction. "Our research suggests that testing chemical compounds that reverse chromatin remodeling is a promising approach to seeking treatments for drug abuse. This is already a major strategy for cancer therapy development," says Dr. Nestler.

GENES REGULATE CRUCIAL PROTEINS

Cocaine activates the genes that provide the templates for building the proteins cFos, ΔFosB, BDNF, and Cdk_5, among others. Researchers have linked the resulting higher brain levels of some of these proteins with long-term consequences of chronic drug abuse. For example, accumulation of long-lasting ΔFosB correlates with cocaine craving and drug self-administration in animals, and may contribute to longlasting structural changes in cocaine abusers' brain reward systems. As researchers continue to trace out the consequences of cocaine-induced gene activation, Dr. Nestler and colleagues pursued a related inquiry: How does it happen? Their candidate explanation was chromatin remodeling, a basic mechanism cells use to alter levels of the body's vast array of proteins to suit new circumstances and challenges (see "Experience Restructures Chromatin").

Chromatin consists of the deoxyribonucleic acid (DNA) double helix that carries an organism's genes wrapped around complexes of histone proteins. The unit of chromatin is called the nucleosome, and chemical processes control how tightly packed nucleosomes are. Chromatin remodeling occurs when this packing becomes more or less compact. As the nucleosomes bunch up or spread out, some genes move into positions that increase—and others into positions that decrease—their ability to interact with RNA polymerase, the enzyme that executes the first step in protein building. This helps determine how much of the protein blueprinted by each gene will be made.

To test their hypothesis that cocaine activates genes by inducing chromatin remodeling, Dr. Nestler and his team compared tissue taken from the striatum of rats exposed to the drug and others given saline. Specifically, they assayed the tissue for the end products of two chemical reactions known to modify chromatin's shape: acetylation and phosphoacetylation of its primary molecular components, histone $_3$ (H_3) and histone $_4$ (H_4). Both of these reactions remodel chromatin in ways that increase gene expression.

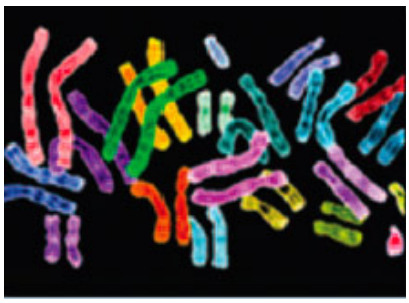
The findings bore out the hypothesis. Within 30 minutes of a single injection, the chromatin associated with the cFos gene in the cocaine-exposed animals contained twice as much acetylated H_4 than that in the control animals, and phosphoacetylated H_3 also was higher. The time course of these effects jibed with previous observations that cocaine induces a rapid, transient increase in levels of the cFos protein. They were no longer present in tissues taken 3 hours after the injection, and they stopped occurring when animals were given repeated cocaine doses over an extended period. These data support scientists' conception of the cFos gene as an early responder to acute neural disruptions, with little or no direct role in situations of recurrent disruption.

As with *cFos*, a single cocaine injection elevated acetylated H_4 in chromatin linked to the *FosB* gene, but the levels returned to baseline within 3 hours. Repeated cocaine did not induce H_4 acetylation in *FosB* geneassociated chromatin, but did cause H_3 acetylation. The researchers say that the switch from H_4 acetylation after a single cocaine exposure to H_3 acetylation after chronic exposure may mark a turning point in developing addiction.

Experience Restructures Chromatin

Chromosomes (pictured) are very long, continuous pieces of DNA that contain genes that help determine an individual's identity. Humans have 23 chromosome pairs with an estimated 30,000 genes.The DNA sequence wraps around proteins that give the chromosome a structure; together, they form chromatin. Cocaine and other external agents and experiences can alter the configurations of these proteins. Depending

on the type of chemical change, the chromatin either bunches up or stretches out, activating or silencing genes along the DNA sequence.



Chromatin reshaping seems to underlie healthy adaptations such as learning and memory as well as disease processes—including cancer, seizures, schizophrenia, and depression. In another study, for example, Dr. Nestler's team found that social stress turned on a particular gene in the brains of mice through chromatin remodeling, a long-lasting change that corresponded with a behavioral indicator of depression. Antidepressant medication reversed both the behavioral sign of depression and the elevated gene activity, underscoring a key point about the modifications: experience and chemical agents can alter gene expression through chromatin remodeling, but such changes are reversible.

"More research is needed to identify the specific molecular basis of this switch," says Dr. Nestler. "However, prior work in my laboratory and with collaborators is starting to fill in a picture of why H_3 acetylation and *FosB's* activation and subsequent triggering of ΔFosB after chronic cocaine might be important. We believe that this series of molecular events, and probably others, mediate the long-term behavioral and neural changes that underlie the transition from drug abuse to addiction," says Dr. Nestler. The experiments and assays also showed:

- A single cocaine injection did not affect *BDNF* or *Cdk5* gene-associated chromatin, but chronic exposure induced H_3 acetylation of both. Once initiated, the effects were long-lasting. The quantities of modified H_3 in *BDNF* gene-associated chromatin in exposed animals increased from 3-fold of those of salinetreated animals at day 1 to 14-fold at day 7. Acetylated H_3 related to the *Cdk5* gene were more than two-fold those of saline 1 day after the last injection, and started to return to control levels only 7 days after cocaine cessation. Such persistent and robust gene activation long after the last dose of cocaine is striking in contrast with the relatively short-lived activation observed for *cFos* and *FosB*.
- Elevations in the ΔFosB protein selectively activated *Cdk5*—the only gene examined in the study that was turned on in this way. This finding suggests that ΔFosB may influence histone modifications by recruiting the chemical agents of chromatin remodeling to some target genes.

"Understanding how cocaine turns on these genes could help addiction researchers develop potential treatments that counteract the effects of drug abuse at the molecular level. Agents that reverse chromatin remodeling are available, and we are examining whether they block cocaine's cellular effects," says Dr. Nestler.

"Taken together with other studies showing that drugs induce long-term structural changes to brain cells, Dr. Nestler's findings show that chromatin remodeling is one way that such neural modification might occur. Such alterations are not necessarily permanent, and studies are needed to determine whether abstinence or other behavioral modifications further restructure chromatin to a state similar to that seen prior to drug exposure," says Dr. Joni Rutter of NIDA's Division of Basic Neuroscience and Behavioral Research. Whether nonstimulant drugs of abuse also act through chromatin remodeling is another important area for future research, she says.

A CONNECTION WITH COCAINE-RELATED BEHAVIOR

In other experiments, Dr. Nestler and colleagues linked chromatin remodeling to cocaine's behavioral effects by examining its role in a laboratory stand-in for human cueinduced drug seeking called conditioned place preference (CPP). By exhibiting CPP—lingering in a part of a cage where it has received a drug—an animal indicates that it is seeking more of the drug (see "Animal Experiments in Addiction Science"). The researchers hypothesized that augmenting or preventing histone modifications during drug administration sessions would increase or decrease CPP, respectively.

COCAINE TURNS ON GENES BY ALTERING CHROMOSOMAL PROTEINS

The researchers found chemical modifications to histone 3 (H_3) and histone 4 (H_4)—major proteins that form the structure of chromosomes—at areas linked with four genes. Acetylation of H_3 and H_4 and phosphoacetylation of H_3 alter the proteins' chemical structure, facilitating gene activation.

Experiment	Gene	Results					
		30 min.	1 hr.	90 min.	3 hr.	1 day	7 days
Acute Cocaine Rats received a single injection, either saline or cocaine (20 mg/kg).	*cFos*	Acetylated H_4 Phosphoacetylated H_3			Normal Histones	Normal Histones	---
	FosB	Acetylated H_4					
	BDNF	No change					
	Cdk5	No change					
Chronic Cocaine Rats received an injection of either saline or cocaine (20 mg/kg) daily for 7 days.	*cFos*	No change					
	FosB	Acetylated H_3		Normal Histones			
	BDNF	Acetylated H_3					Normal Histones
	Cdk5	Acetylated H_3					Normal Histones
Cocaine Withdrawal Rats received an injection of either saline or cocaine (20 mg/kg) daily for 7 days and did not receive the drug again.	*cFos*	Effects too transient to examine					
	FosB	Effects too transient to examine					
	BDNF	Acetylated H_3: Levels rise to 3-fold those associated with saline injection after 1 day, and to 14-fold after 7 days.					
	Cdk5	Acetylated H_3: Levels rise to 3-fold those associated with saline injection after 1 day, and return to normal after 7 days.					Normal Histones

The investigators administered cocaine to mice daily for 4 days. Before each administration, they treated one group with a drug that enhances histone acetylation (trichostatin A, TSA) and another with a virus that expresses an enzyme that blocks this particular modification (herpes simplex virus, HSV). When placed back in the test cage on day 5, the group given TSA doubled the time spent in the drug-associated cage, on average, relative to the control

group. In contrast, the group given the HSV vector lingered in the test cage for one-third of the time spent by its control group.

The findings suggest a causal link between histone acetylation in the striatum and sensitivity to cocaine's behavioral effects.

"The team had already demonstrated that chromatin remodeling plays a role in the rewarding aspects of cocaine abuse by including a group of animals that selfadministered the drug in the study. Their CPP experiment further strengthens the connection between histone restructuring and behavioral aspects of addiction and suggests that agents that reverse chromatin restructuring hold promise as potential therapies," says Dr. Rutter.

SOURCE

Kumar, A., et al. Chromatin remodeling is a key mechanism underlying cocaine-induced plasticity in striatum. *Neuron* 48(2):303-314, 2005. [Full Text]

Volume 21, Number 4 (October 2007)