Serotonin System May Have Potential as a Target for Cocaine Medications

By targeting specific receptors of the neurochemical serotonin, investigators hope to advance the development of potential relapse prevention agents.

BY LORI WHITTEN, NIDA Notes Staff Writer

NIDA-supported researchers have weakened rats' behavioral responses to environmental cocaine cues by manipulating the neurotransmitter serotonin. Moreover, such manipulation can make the drug seem less stimulant-like to the rats. The findings suggest that medications that act on the serotonin system might help recovering cocaine abusers sustain abstinence, say Dr. Kathryn Cunningham and colleagues at the Center for Addiction Research at the University of Texas Medical Branch in Galveston and the Polish Academy of Sciences in Krakow.

While cocaine makes its primary pharmacological impact on the neurotransmitter dopamine, it also increases levels of other chemical messengers, including serotonin (5-HT). Previous research with animals has shown that 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors—two proteins on brain cell surfaces that mediate serotonin's effects on cellular activity—regulate behavioral responsiveness to cocaine. For example, activating the 5-HT$_{2C}$ receptor reduces the animals' typical behavioral responses to cocaine—including hyperactivity, self-administration, and return to drug-seeking following abstinence. Dr. Cunningham's studies showed that the 5-HT$_{2C}$ receptor affected responsiveness to cocaine-associated environments, and that both receptors affected the animals' experience of the drug.

HYPERACTIVITY AND DISCRIMINATION

Dr. Cunningham and colleagues first examined the effect of manipulating 5-HT$_{2C}$ receptors on a behavior called conditioned hyperactivity: When researchers repeatedly move an animal from its home cage and give cocaine in a test cage, the drug-paired environment comes to evoke the same behavioral effect as the stimulant itself when saline is administered, so that the animal starts moving about restlessly as soon as it finds itself in the cage. Experience has shown that compounds that inhibit conditioned hyperactivity usually also reduce behaviors that are laboratory stand-ins for human relapse.

The Drug Discrimination Protocol

In their second experiment, Dr. Cunningham and colleagues used a
drug discrimination procedure to determine whether the test compounds changed how cocaine felt to the animals. To each rat, they first gave randomly alternating infusions of cocaine and saline. After each infusion, the rat could obtain a water reward by pressing Lever A if it had received cocaine or Lever B if it had received saline. After numerous repetitions, the rat regularly pressed the correct lever—demonstrating that it had learned the challenge, wanted the reward, and could discriminate between the experiences produced by cocaine and saline. Next, the researchers observed the rat's performance when it was given a receptor blocker prior to cocaine:

- Rats given a $5\text{-HT}_{2A}$ receptor blocker no longer went as reliably to Lever A, indicating that they were less able to distinguish cocaine from the placebo;
- Rats given a $5\text{-HT}_{2C}$ receptor blocker pressed more on Lever A, indicating enhanced cocaine-like effects.

Dr. Cunningham and colleagues administered cocaine (15 mg/kg) to rats daily for 7 days in a test cage. Two days later, they gave some of the rats a compound that activates the $5\text{-HT}_{2C}$ receptor (MK 212), some a compound that blocks it (SB 242084), and others saline, and returned the animals to the test cage. Compared with the saline-treated animals, who showed the usual conditioned hyperactivity, rats given the $5\text{-HT}_{2C}$-receptor-stimulating compound moved around less (by about 40 percent), while those that received the blocker showed an exaggerated hyperactive response (by 25 percent) to the test cage. A separate group of animals was given cocaine only in their home cage and saline in the test cage. These animals showed normal activity when tested 2 days later, which was unaffected by the $5\text{-HT}_{2C}$ receptor compounds. These data strengthen the conclusion that the $5\text{-HT}_{2C}$ receptor is important in the cocaine-environment link.

In another study, Dr. Cunningham's team used an experimental protocol called drug discrimination to determine whether compounds that act at the $5\text{-HT}_{2C}$ and $5\text{-HT}_{2A}$ receptors would alter the way cocaine made the rats feel (see textbox, below). Prior research had indicated that the two receptors oppose each others' effects on the cocaine response, and the researchers hypothesized that blocking the $5\text{-HT}_{2A}$ receptors would make cocaine feel less stimulant-like to the rats, whereas inhibiting $5\text{-HT}_{2C}$ receptors would enhance the drug's effects. Rats given a compound that blocks $5\text{-HT}_{2A}$ receptors (SR 46349B) prior to cocaine reduced their pressing on the lever associated with cocaine's effects compared with one linked with saline. Animals pretreated with a compound that blocks $5\text{-HT}_{2C}$ receptors (SDZ SER-082) increased their pressing on the cocaine lever over the saline lever. The results bore out the hypothesis.

"Taken together, the findings of these studies support the idea that the serotonin $5\text{-HT}_{2C}$ receptor plays a role in linking environmental cues and the experience of cocaine, as well as the subjective effects of the drug. The $5\text{-HT}_{2A}$ receptor also influences these behaviors, but in the opposite direction," says Dr. Cunningham. "From the medication development perspective, a drug with dual action at both receptors—that is, one that simultaneously stimulates the $5\text{-HT}_{2C}$ receptor and blocks the $5\text{-HT}_{2A}$ receptor—might be effective in reducing cue-induced craving. We know of no such compound, and our team is working to develop one." She adds that agents that stimulate $5\text{-HT}_{2C}$ or inhibit $5\text{-HT}_{2A}$ receptors do not fully mimic cocaine or affect other behaviors, suggesting limited
side effects.

More Data Point to Serotonin

Dr. Janet Neisewander and colleagues at Arizona State University confirmed the therapeutic potential of compounds that act on the two serotonin receptors ($\text{5-HT}_{2A}$ and $\text{5-HT}_{2C}$) that Dr. Cunningham's team examined. Using a self-administration-extinction-trigger-exposure model of testing for relapse (see "Animal Experiments in Addiction Science"), the researchers found that:

- **Ketanserin**—a compound that blocks the $\text{5-HT}_{2A}$ and $\text{5-HT}_{2C}$ receptors—attenuated cue-induced relapse to cocaine, but not drug-triggered relapse. Because ketanserin blocks both receptors, the investigators did not know which might have been responsible for preventing cue-induced relapse. By combining data from this experiment and the one described below, the researchers were able to zero in on the $\text{5-HT}_{2A}$ receptor.

- **SB 242084**, a selective blocker of $\text{5-HT}_{2C}$ receptors, did not affect cue-induced or cocaine-triggered drug-seeking. The findings suggested that the results seen with ketanserin most likely were due to its ability to block the $\text{5-HT}_{2A}$ receptor. The researchers concluded that blocking the $\text{5-HT}_{2A}$ receptor might help prevent relapse triggered by environmental cues associated with taking cocaine, the same inference as Dr. Cunningham's team.

In another experiment, Dr. Neisewander's team found that SB 242084 interfered with the ability of a drug that augments serotonin, $d$-fenfluramine, to prevent cue-induced cocaine seeking. Because blocking the $\text{5-HT}_{2C}$ receptor made $d$-fenfluramine ineffective, the investigators concluded that stimulating the receptor may help prevent cue-induced relapse.

The compounds tested by Dr. Neisewander's team will not necessarily be developed as medications to prevent cocaine relapse, but the results of the study do suggest that drugs that act on serotonin may be potential pharmacotherapy candidates. The findings add to a growing number of studies that suggest the promise of a dual-action pharmacotherapeutic approach for relapse prevention—that is, a drug that simultaneously blocks $\text{5-HT}_{2A}$ receptors and stimulates $\text{5-HT}_{2C}$ receptors.

"Recent evidence suggests that serotonin is involved in motivation for various pleasurable experiences, including food. Researchers developing medications for obesity also are studying the effect of stimulating the 2C receptor, which may be a point of intersection for several addictions," says Dr. Cunningham.


"It is not really surprising that serotonin is implicated in addiction given its importance to essential behaviors—including sleep, eating, mood, cognitive processes, and self-regulation—and its influence on dopamine," says Dr. Minda Lynch of NIDA's Division of Basic Neuroscience and Behavioral Research.
"Serotonin influences dopamine in the brain's reward pathway and cortex, so examining the behavioral effects of serotonin-influencing compounds in animals is a reasonable approach in the investigation of potential pharmacotherapies," says Dr. Lynch. She agrees that a dual-action compound that operates on the serotonin 5-HT\textsubscript{2C} and 5-HT\textsubscript{2A} receptors might eventually help prevent relapse. "However, a great deal of further testing in animals is needed. A good next step would be to confirm the findings in animal protocols that mimic cue-induced relapse," says Dr. Lynch.

**SOURCE**


Behavioral Response to Novelty Foreshadows Neurological Response to Cocaine

Young rats' engagement with novel objects correlates with cocaine-induced dopamine release, shedding light on the mechanisms of drug abuse vulnerability.

BY LORI WHITTEN, NIDA Notes Staff Writer

NIDA-supported researchers Dr. Cheryl Kirstein and Ms. Kirstie Stansfield at the University of South Florida have found that higher scores on tests of impulsivity and some behavioral responses to novelty correlate with a heightened biological response to cocaine in adolescent, but not adult, rats. The findings accord well with scientists' widely shared view that developmental differences in brain systems that use the neurotransmitter dopamine underlie age differences in susceptibility to drug abuse.

Dr. Kirstein and Ms. Stansfield conducted a series of behavioral assays to rate rats' relative responsiveness to novelty, then compared these results with measures of dopamine release in the reward pathway after an injection of cocaine. First, they put adolescent rats (34 days old, which is roughly equivalent to adolescence in people) and fully mature rats (59 days old, equivalent to human young adulthood) through four behavioral protocols. The tests measured activity in a new environment (how much the rat moved around when put into a new cage); impulsivity (how quickly it approached a new object placed into its cage); exploratory drive in response to a new object (how many times it approached the object in a given period of time); and attraction to new objects (what percentage of a given time interval was spent close to the object).

The researchers then injected the animals with saline and then, 2 hours later, with cocaine 20 mg/kg. Every 10 minutes, starting immediately after the saline injection and continuing until 2 hours after administering the cocaine, they measured the concentrations of the neurotransmitter dopamine and its major metabolite in the rats' nucleus accumbens (NAc). The measurements were made using the technique of in vivo microdialysis. By the time of the last
measurement, the drug had cleared the animal's system.

**ON MOST TESTS, AGE MATTERS**

In their analysis, the researchers compared cocaine-induced dopamine release in animals that had responded above the mean level on each test (high responders, HR) to those who had scored below the mean (low responders, LR). The results revealed that among both the adult and adolescent rats, those that exhibited greater activity in a new environment also demonstrated enhanced dopamine release following a cocaine injection. This was the only test, however, in which age did not influence cocaine-induced dopamine release. The other behavioral assays revealed interactions between age and the response to novelty on cocaine-induced dopamine release in the NAc:

- Impulsivity—Adolescent rats with above-the-mean impulsivity scores released more dopamine in response to cocaine than their age mates who were LR. Mature rats exhibited no clear relationship between impulsivity and cocaine-induced dopamine response.
- Exploration of a new object—Adolescent rats with above-the-mean scores on this measure released more dopamine in response to cocaine than their age mates who were LR. Adult rats showed the opposite pattern: Animals with above-the-mean scores showed attenuated cocaine-induced dopamine release compared with age mates who were LR.
- Attraction to a new object—Adolescent rats exhibited no clear relationship between reactivity on this assay and cocaine-induced dopamine release. Mature rats with above-the-mean scores released less dopamine in response to cocaine compared with their age mates who were LR.

Dr. Kirstein's finding that for all the animals, greater activity in a new environment corresponded with increased sensitivity to stimulants is consistent with earlier research. Her team's mixed findings on the impulsivity and other novelty response tests indicates, she says, that those behaviors arise from different physiological mechanisms than does locomotor activity. "My colleagues and I think locomotor activity may reflect primarily dopamine activity in a brain circuit involved with generating and controlling movement. Novelty may instead differentially stimulate mesolimbic dopamine—a pathway implicated in attention as well as reward and motivation," says Dr. Kirstein.

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**In Vivo Microdialysis**

The investigators used *In Vivo* microdialysis to measure dopamine each animal released from its nucleus accumbens (NAc) in response to cocaine. They implanted a probe into the shell area of the NAc. The probe is a fine tube, about the size of a sewing needle, connected to a mini-pump that continuously perfuses it with artificial cerebrospinal fluid. The membrane tip of the probe captures dopamine and its metabolites. The samples collected by the needle are then analyzed using techniques, such as chromatography, that are able to isolate dopamine and its metabolites from other molecules.

**INHIBITION DEVELOPS LATER**

The findings on the three tests where age affected the relationship between behavior and cocaine-induced dopamine release may reflect maturation of the brain's reward circuit. When rats are adolescents, dopamine-producing and releasing cells in this circuit may be particularly sensitive both to novelty and to pharmacological stimulation. As part of normal neurological development, areas of the brain that dampen the activity of this circuit come "online" later, explaining the age-related differences observed in Dr. Kirstein's study. "The
The mesolimbic pathway and the cortical areas that inhibit it to regulate dopamine release are not yet fully matured in the adolescent, and this may explain why the adolescent brain responds to drugs differently than the adult brain," says Dr. Kirstein.

"The results of Dr. Kirstein's study, along with other animal research on the interaction of drugs and developmental stage, indicate that the adolescent brain is more responsive to drugs than the adult brain—both neurochemically and behaviorally," says Dr. Nancy Pilotte of NIDA's Division of Basic Neuroscience and Behavioral Research. Studies that identify the physiological and behavioral processes underlying age-related susceptibility to addiction complement epidemiological work on the individual and social factors contributing to adolescent vulnerability to substance abuse.

**SOURCE**

Cocaine Craving Activates Brain Reward Structures; Cocaine "High" Dampens Them

A study documented changing emotional and neurobiological responses to cocaine with successive doses during a single session of drug taking.

BY LORI WHITTEN, NIDA Notes Staff Writer

NIDA-funded researchers mapped the dynamic of drug-induced brain activity and emotional responses that occur during a cocaine abuser's typical binge-like pattern of self-administration. Dr. Robert Risinger and colleagues at the Medical College of Wisconsin found that craving corresponds with increased activity in key brain areas underlying reward and motivation, while the cocaine-induced "high" is linked with decreased activity in these same regions. "Our results suggest that, as one takes multiple 'hits' of cocaine, pleasure accumulates with each successive dose, but lasts for a shorter time—a pattern that would compel people to keep abusing," he says.

"My colleagues and I wanted to know what cocaine does to the brain to compel drug-seeking behavior in addicted people—particularly, why taking a small amount of the drug can lead to a binge. Understanding this could help identify interventions to stop such abuse," says Dr. Risinger.

Dr. Risinger's team recruited six cocaine-addicted men who were not seeking treatment. The men, aged 23 to 41, had abused crack cocaine for 11 years, on average. They completed a medical examination, received counseling on the health consequences of cocaine abuse, and were offered (but all declined) addiction treatment before the study.

Each man participated in two 1-hour sessions of cocaine self-administration. At the beginning of the first session, he learned how to press a joystick button to receive infusions of cocaine through an intravenous catheter. After a 5-minute baseline period, he saw a computer-displayed signal that the

COCAINE CRAVING AND "HIGH" CORRESPOND WITH OPPOSITE PATTERNS OF BRAIN ACTIVATION

Cocaine craving was linked with activation (red areas, top panel), and euphoria with deactivation (blue areas, bottom panel) of the same brain regions according to an fMRI study in which cocaine-addicted participants reported subjective responses during a 1-hour self-administration session. The brain regions affected by craving and high are involved in reward anticipation, emotional response, and control over actions. The insula—a brain structure that seems to translate bodily sensations into emotions—was activated during cocaine-induced euphoria (red area, bottom panel).
joystick was activated, and for the next 55 minutes he pressed the button at will. Each press delivered a 20 mg/70 kg of body weight dose of the drug—except that, for safety reasons, doses could not be repeated at intervals of less than 5 minutes, and total doses over the course of the hour were limited to six. Meanwhile, in response to prompts on a computer display, the volunteer used a joystick to rate his cocaine craving, high feelings, and other sensations once per minute. During the second session, the researchers used functional magnetic resonance imaging (fMRI) to obtain brain scans synchronized with the subjective reports. After each session, each man underwent a brief physical examination and left the facility once his vital signs returned to baseline levels and he no longer showed drug effects or reported craving.

BEHAVIORAL RESULTS

Participants administered 4.5 injections a session, on average, spacing the doses about 7.4 minutes apart. Only two administered the maximum six doses. "For many patients, the amount of cocaine consumed during a self-administration session was less than they typically abused," says Dr. Risinger.

As anticipated, the men's feelings of being high decreased before and increased after cocaine administration. From the first through the fourth injection, the intensity of each successive high was greater, and its duration shorter. Craving peaked about 1 minute before each injection and decreased to a low point about 2 minutes after cocaine administration, before rising again during minutes 3 to 4. Absolute levels of craving decreased with each successive injection, but the pre-administration increase in craving rose more sharply.

Dr. Risinger says the participants' reports match other abusers' accounts of their feelings during binges: "People often talk about 'chasing the high.' They abuse the drug several times in an episode, feel increasingly high with the first few hits, and experience a rapid dropoff in the duration of pleasure with repeated use—which may explain consuming larger amounts and more frequently over a session. Consuming cocaine satisfies craving only briefly, and then the feeling increases again before another administration, which may also contribute to the binge pattern."

"It is not clear whether the subjective feeling of craving was directly responsible for driving the participants to self-administer, or whether some other process, perhaps response-outcome learning, was responsible for initiation of self-administration," says Dr. Steven Grant of NIDA's Division of Clinical Neuroscience and Behavioral Research.

IMAGING FINDINGS
Cocaine-induced craving was associated with increased neural activity in brain areas involved in reward anticipation, emotional response, and control over actions: the nucleus accumbens (NAc), the orbitofrontal cortex, and the anterior cingulate cortex. The findings accord well with those of cue-induced craving studies, which generally indicate that the anticipation of a reward is accompanied by activation of the dopamine-rich mesolimbic pathway—a neural circuit involved in reward, motivation, and directing attention to stimuli. Such a neural response is thought to "set up" the brain to experience reward and to drive goal-directed behavior.

The study represents an important step in correlating drug-induced craving and high with neural activity in specific regions of the human brain. "It provides insight into the neurobiology involved in drugtaking binges, a very common and dangerous behavior associated with the disease of addiction," says Dr. Risinger. "Dr. Risinger's study is a good example of translational research, which applies a well-established technique in animal research to people. Although the results need replication in a larger number of participants, the findings are provocative because they raise good questions about the relationship between the various neurobiological responses—the fMRI signal and dopamine release, for example. We currently do not have a complete picture of how neurochemical responses and neural activation patterns exactly relate to the entire drug-taking experience, but this issue can be addressed in reverse translational research—animal imaging studies of self-administration and passive cocaine delivery," says Dr. Grant. NIDA-funded investigators are developing such techniques, he adds.

**SOURCES**


[Abstract]