Research Findings
Vol. 20, No. 2 (August 2005)

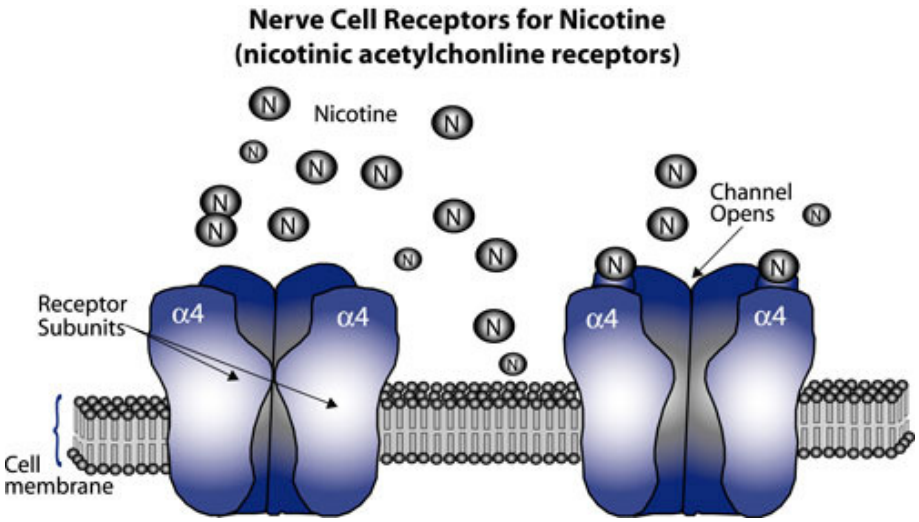
Site on Brain Cells Appears Crucial to Nicotine Addiction

By Patrick Zickler, *NIDA NOTES* Staff Writer

Using genetic engineering, NIDA-supported scientists have produced a strain of mice with special characteristics that can help researchers identify and study key steps in the development of nicotine addiction. By altering a single amino acid in just one of a mouse's 30,000 genes, the scientists produced mice that are exceptionally sensitive to the effects of nicotine. The modified mice show behaviors associated with addiction when exposed to nicotine doses far too small to cause similar effects in other mice. Their dramatically increased sensitivity suggests that the brain cell site affected by the modified gene is crucial to development of nicotine addiction.

Dr. Andrew Tapper and colleagues at the California Institute of Technology in Pasadena and at the University of Colorado in Boulder built on work by other scientists which indicated that a site on some brain cells—the α4 subunit of nicotine receptors—plays a key role in the brain's response to nicotine. The previous work involved "knock-out" mice, in which scientists had disabled a gene that directs development of the α4 site. When exposed to nicotine, the α4 knock-out mice did not respond with increased release of the pleasure-causing brain chemical dopamine, a reaction thought to be a key factor in the development of nicotine addiction.

Brain Pathway to Nicotine Addiction



Nicotine attaches to nerve cells in the brain at receptors on the cell membrane. The receptors comprise five subunits that fit together like sections of an orange. When a nicotine molecule binds to one of these subunits, the segments pull away from each other, creating an open channel through the cell membrane. This initiates a series of electrical and chemical signals that trigger release of dopamine by other brain cells. One type of subunit, designated α4, appears to play a central role in development of nicotine addiction; mice engineered to have especially sensitive α4 subunits exhibit behaviors characteristic of nicotine addiction when exposed to a dose of nicotine just one-fiftieth of that normally needed to elicit these behaviors.

The results with knock-out mice suggested that α4 sites on brain cells are necessary for development of nicotine addiction, but didn't address the question of whether the sites are sufficient by themselves to initiate the behaviors associated with addiction. To answer that question, says Dr. Henry Lester of the California Institute of Technology, "We decided to create animals with hypersensitive α4 receptors. That way, instead of eliminating the response to nicotine, we could emphasize it and study the processes that lead to nicotine addiction. So we developed the α4 'knock-in' mouse."

The scientists compared the behavioral effects that are in part characteristic of nicotine addiction—reward, tolerance, and sensitization—in their knock-in mice and unmodified mice. According to Dr. Lester, the results indicate that activation of the α4 site by nicotine is sufficient to initiate the effects.

Reward: The researchers measured nicotine reward in their mice with a technique called "conditioned place preference," which is based on the assumption that if animals like an experience, such as receiving nicotine, they will gravitate to the place where they have had that experience rather than another where they haven't. In the experiment, mice with unmodified α4 receptors exhibited a preference for a compartment associated with a nicotine dose of 0.5 mg/kg of body weight—a typical dose ingested by a human smoker. The investigators then tested the rewarding effect of one-fiftieth of that amount, 10 µg/kg, on the unmodified and the α4 knock-in mice. When allowed to move freely between the chambers for 20 minutes following nicotine administration, the unmodified mice showed no preference for the nicotine-associated compartment; they spent slightly less time in that chamber than they had before. In contrast, modified mice showed a marked preference for the compartment associated with nicotine, spending an average of 2 minutes more in that chamber following nicotine administration.

Tolerance and sensitization: To test tolerance to nicotine, the investigators subjected the unmodified and knock-in mice to repeated doses of nicotine, 15 µg/kg daily over 9 days, and then compared the changes in nicotine-induced hypothermia. The unmodified mice showed no change in body temperature, but the knock-in mice exhibited a decrease of 3°C on the first and second days, and smaller decreases each successive day, suggesting they had developed tolerance to the nicotine-induced hypothermia. In tests for sensitization, only the genetically engineered mice increased activity levels (measured by counting the number of times the animals cross a beam of light in the 60 minutes following injection) in response to daily injections of 15 µg/kg over 9 days.

"This work represents a significant step forward in understanding how nicotine hijacks the brain's normal signaling process," says Dr. Joni Rutter of NIDA's Division of Basic Neuroscience and Behavioral Research. "And the research approach—moving from manipulation of a single protein to an animal's behavioral response to nicotine—also holds great promise. If the α4 site is also found to play a large role in human nicotine addiction," Dr. Rutter adds, "it is a promising focus for research into medications that might block nicotine's effects."

Source

- Tapper, A.R., et al. Nicotine activation of α4 receptors: Sufficient for reward, tolerance, and sensitization. *Science* 306(5698):1029-1032, 2004. [Abstract]

Genetic Engineering Reveals Proteins' Key Role in Sensitivity to Cocaine

Genetic engineering strategies like those used at the California Institute of Technology to study nicotine addiction have helped other investigators identify a pair of proteins that seem to influence cocaine addiction.

Dr. Peter Kalivas and his colleagues at the Medical University of South Carolina in Charleston developed a strain of mice lacking two genes, called *Homer1* and *Homer2*, that direct production of proteins linked to cocaine's effects in the brain. The researchers found that the *Homer* "knock-out" mice were more sensitive than unmodified mice to the behavioral effects of cocaine.

Compared with unmodified mice, animals missing either *Homer1* or *Homer2* developed stronger place conditioning—when allowed to move freely, they would spend more time in a compartment where they had received cocaine than in a compartment with no drug association. The knock-out mice also were more sensitive to cocaine's stimulatory effect; when placed in a chamber equipped with photoelectric beams that could measure activity, the knock-outs were approximately 50 percent more active than unmodified mice following cocaine injections. To verify the role of the *Homer* genes in increased sensitivity to cocaine, the researchers restored *Homer* genes in the brains of the knock-outs, eliminating the previously seen differences in stimulation and place conditioning.

"The fact that *Homer* deletions result in these augmented responses to cocaine suggests that disruption of *Homer* protein-regulated signaling in the brain is a central step in development of cocaine addiction," Dr. Kalivas says. Additional evidence of this role is seen in changes that *Homer* deletion causes in levels of the brain messenger chemical glutamate, he adds. *Homer* knock-out mice that had never been exposed to cocaine had nucleus accumbens (NAc) glutamate concentrations about 50 percent lower than mice with the genes—an effect similar to that seen in mice after cocaine withdrawal. This effect, too, was reversed when the scientists injected *Homer* genes into the NAc.

The association between *Homer* activity and the conditions of cocaine withdrawal is particularly intriguing, according to Dr. Kalivas, because other researchers have shown that *Homer* protein levels rise and fall in response to environmental cues and changing levels of stress. "*Homer* may be a window to study the molecular basis of the important link between environmental stress and cocaine addiction."

Source: Szumlinski, K.K., et al. *Homer* proteins regulate sensitivity to cocaine. *Neuron* 43(3):401-413, 2004. [Full Text]

Volume 20, Number 2 (August 2005)

Research Findings
Vol. 20, No. 1 (August 2005)

Researchers Investigate Cocaine "Abstinence Syndrome"

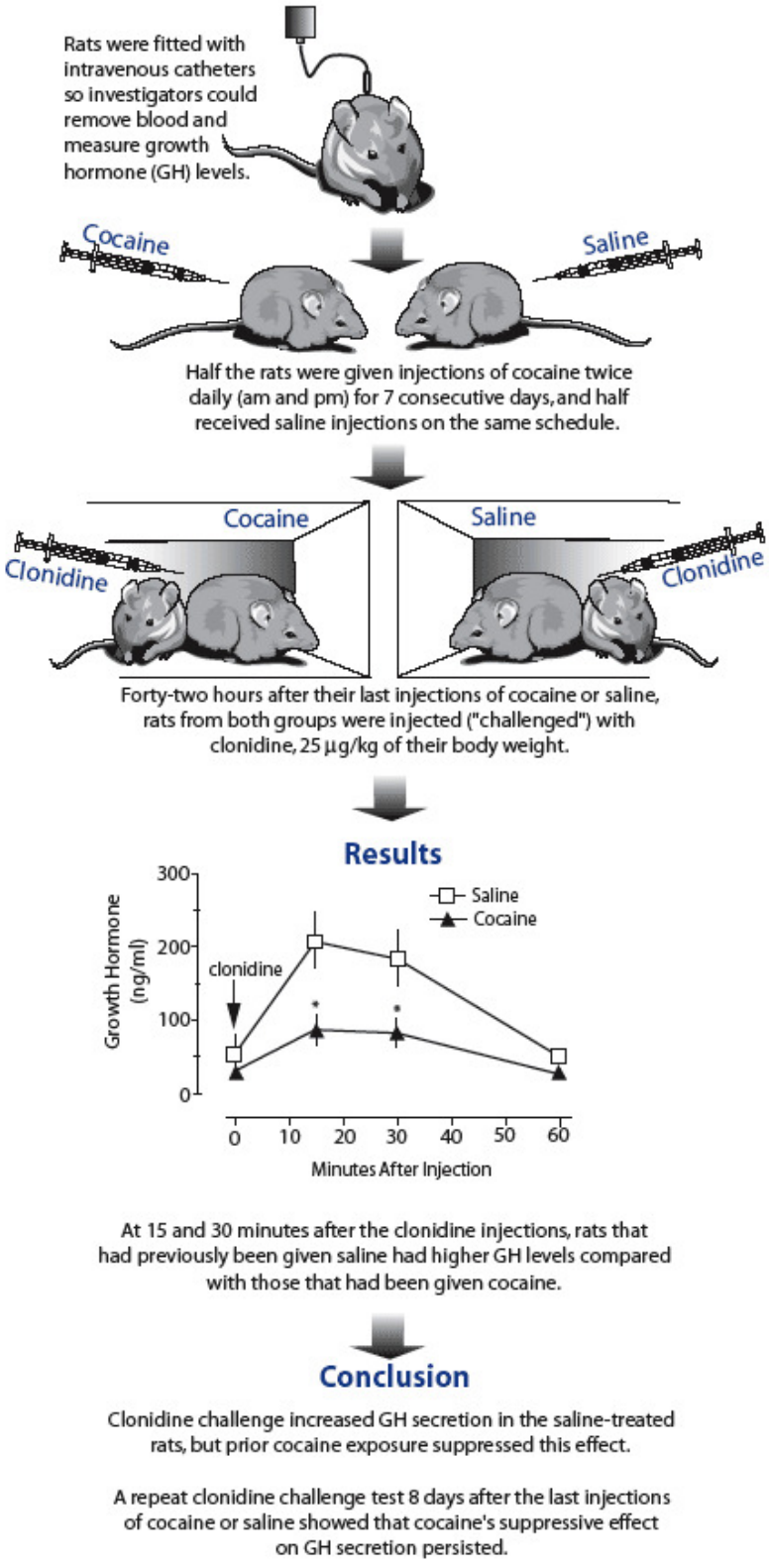
By Lori Whitten, *NIDA NOTES* Staff Writer

Researchers have long focused on motivation as the centerpiece of the addiction puzzle, based on the observation that in many addicted individuals, compulsive drug-seeking behavior overtakes the most fundamental motivators, including food and sex. Now, however, researchers are beginning to examine another aspect of addictive drugs—their powerful and long-lasting effects on mood. People who have recently stopped abusing stimulant drugs commonly experience "abstinence syndrome": low energy, irritability, restlessness, an inability to feel pleasure, and problems with concentration. Anxiety and panic attacks also are sometimes associated with cocaine abstinence. Addiction researchers are examining the neurobiology underlying abstinence syndrome with an eye toward improving current therapies' ability to alleviate these symptoms and prevent relapse.

NIDA investigators concentrated recently on the impact of cocaine on the neurotransmitter norepinephrine (NE), one of the two neurochemicals most responsible for mood. Stimulation of brain cells by serotonin and NE is central to positive mood, feeling energetic, and maintaining focus as well as sleep, appetite, and coping with stress. Two recent animal studies, one conducted by investigators at NIDA's Intramural Research Program (IRP) in Baltimore and another by NIDA-funded researchers at Harvard Medical School's New England Primate Research Center in Southborough, Massachusetts, suggest that cocaine may compromise NE's ability to stimulate brain cells by altering a communication protein, called the α2-adrenergic receptor, on the surfaces of the cells.

In the Baltimore study, Dr. Michael Baumann and colleagues hypothesized that by giving rats cocaine regularly—twice-daily injections at 15 mg/kg of the animals' body weight for 7 days—and then abruptly stopping it, they would reduce the α2-adrenergic receptors' responsiveness. To assess the adrenergic system in the cocaine-exposed, now "abstinent" rats, the researchers used the clonidine challenge procedure, which indirectly indicates α2-adrenergic receptor activity by measuring how much plasma levels of growth hormone (GH) increase following exposure to the drug clonidine (see "Clonidine Challenge Suggests That Cocaine Abuse Desensitizes Adrenergic System"). Confirming the researchers' hypothesis, the cocaine-exposed animals showed a blunted GH response—less than half that of saline-exposed animals—15 and 30 minutes after the clonidine challenge. The rats' response was still low, but returning toward normal, when the researchers repeated the challenge procedure 8 days after daily injections stopped.

Clonidine Challenge Suggests That Cocaine Abuse Desensitizes Adrenergic System



Clonidine elevates growth hormone (GH) secretion in rats, a response mediated through the a2-adrenoreceptors in the brain. When rats are exposed to repeated cocaine injections, GH secretion in response to clonidine is lowered. The clonidine challenge test is used to measure a2-adrenoreceptor sensitivity in humans as well as animals.

The findings suggest that cocaine consumption and cessation may lower recovering individuals' moods by desensitizating the α2-adrenoreceptors, but the results are preliminary. "The adrenergic system is complex, with multiple pathways in the brain and body," says Dr. Baumann. "We still have much to learn about how drug exposure affects all these pathways, how it affects serotonin, and how they both influence growth hormone."

People with depression secrete less GH in response to the clonidine challenge than do those without the condition, a clinical finding that suggests possible links between NE receptor function, mood disorders, and cocaine withdrawal. "Although investigators are only beginning to characterize norepinephrine's role in addiction, a growing body of animal and clinical research suggests important connections between the adrenergic system, mood and anxiety disorders, and the depression-like symptoms experienced by people trying to overcome cocaine addiction," Dr. Baumann says.

A Role in Relapse?

In a study that explored the chemical basis of mood and cocaine relapse, Dr. Roger Spealman and colleagues hypothesized that blocking α2-adrenergic receptors in monkeys would generate anxiety and induce a resumption of previously extinguished cocaine-seeking behavior.

The researchers trained monkeys to seek cocaine by pressing a lever. When the monkeys reached a high rate of lever pressing, the researchers disconnected it from the injection device. The monkeys kept trying the lever for a while, but with no more cocaine forthcoming, gradually left off. The investigators hypothesized that giving monkeys a drug to reduce NE activity would make the animals anxious, and the anxiety would intensify their urge for cocaine to the point where they would resume pressing the lever despite recent experience of its futility.

Dr. Spealman and colleagues gave the now "abstinent" animals various doses of two α2-adrenergic blocking agents, yohimbine and RS-79948, in separate test sessions. Both α2-adrenergic receptor blockers set the animals to pressing the lever again. The increase ranged from 1.5 to 4 times the response to injections of sterile water, depending on the dose and drug. The yohimbine injections also increased physiological and behavioral signs of anxiety: salivary cortisol levels and self-grooming and scratching.

"It makes sense physiologically that the adrenergic system might play a role in addiction; cocaine activates norepinephrine as much as it stimulates dopamine."

To confirm that yohimbine's behavioral effects were due to its inhibition of the α2-adrenergic system, rather than any of the other neurotransmitter systems this agent affects, the researchers conducted further experiments. First, they gave the monkeys yohimbine plus clonidine, a drug that selectively blocks yohimbine's effects on the α2-adrenergic receptors. With this regimen, the animals resumed lever pressing hardly or not at all. Next, the researchers gave the monkeys yohimbine plus flupenthixol, a drug that reduces dopamine activity and has no effect on yohimbine's inhibition of α2-adrenergic activity. Under this regimen, the animals did resume lever pressing. Both of these findings pointed to α2-adrenergic suppression as the key to yohimbine's effects in the first experiment. Yohimbine did not stimulate movement or make the animals restless, which indicates that it worked by blocking receptors, not simply by mimicking the stimulant effects of cocaine.

"It makes sense physiologically that the adrenergic system might play a role in addiction; cocaine activates norepinephrine as much as it stimulates dopamine," says Dr. Spealman. Work by others suggests that cocaine abuse leads to long-term desensitization of the NE system in areas of the brain involved in reward and stressinduced reinstatement of drug-seeking. "This has led some researchers to speculate that the desensitized adrenergic system increases vulnerability to further norepinephrine disturbances—for example, those caused by stress or drug re-exposure—which may increase relapse risk," explains Dr. Spealman.

Researchers continue to seek to unravel the complexities underlying withdrawal and relapse to drug-taking. If, as scientists now think, these phenomena arise from sequential alterations in both the reward and mood pathways, "addiction medications may have to target different neurotransmitters at various stages of abstinence," says

Dr. Minda Lynch of NIDA's Division of Basic Neuroscience and Behavioral Research.

Sources

- Baumann, M.H.; Milchanowski, A.B.; and Rothman, R.B. Evidence for alterations in α2-adrenergic receptor sensitivity in rats exposed to repeated cocaine administration. *Neuroscience* 125(3):683-690, 2004. [Abstract]
- Lee, B.; Tiefenbacher, S.; Platt, D.M.; and Spealman, R.D. Pharmacological blockade of α2-adrenoreceptors induces reinstatement of cocaine-seeking behavior in squirrel monkeys. *Neuropsychopharmacology* 29(4):686- 693, 2004. [Full Text]

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Sigma Antagonists: Potential Cocaine Medications With Novel Activity

Research Findings
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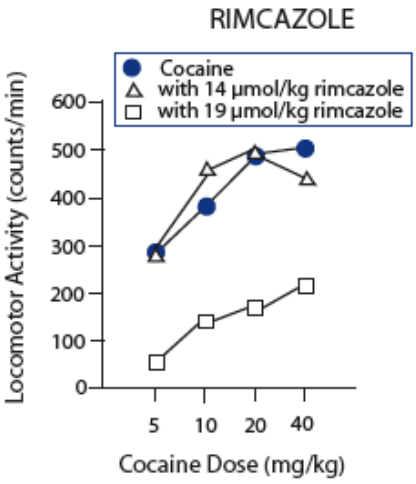
By Patrick Zickler, *NIDA NOTES* Staff Writer

Investigators in NIDA's Intramural Research Program (IRP) have confirmed and extended previous findings that rimcazole, a medication developed in the 1980s to treat schizophrenia, weakens some of cocaine's effects in rodents. The new findings strengthen speculation that drugs like rimcazole might help recovering individuals avoid temptations to relapse to cocaine. Rimcazole and related compounds share a novel mechanism of action, a sigma receptor blockade, that appears to have significant potential for loosening cocaine's hold on addicted individuals.

Coadministration of Rimcazole With Cocaine Blocks Locomotor Stimulation in Mice



When administered with cocaine, rimcazole blocked the stimulant effects of cocaine on locomotor activity.

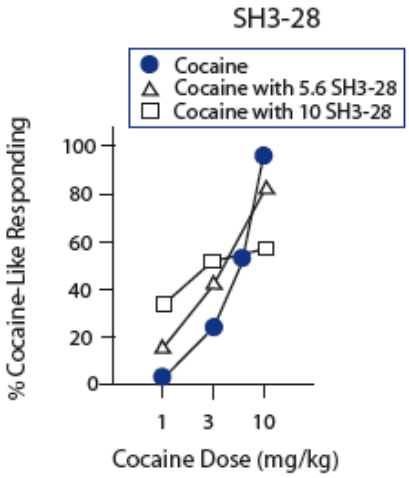
Drs. Jonathan Katz and Amy Newman and IRP colleagues showed that pretreating mice with rimcazole reduced one of cocaine's signature effects on rodent behavior: increases in locomotor activity—more running around. The impact on movement varied with the dosages of rimcazole and cocaine: At the maximum, a 73 µmol/kg dose of rimcazole reduced by 57% the amount of locomotor activity rats exhibited following administration of cocaine (40 mg/kg). Other rimcazole-like compounds reduced locomotor activity following cocaine exposure by up to 47%, also depending on dosages.

The demonstration that rimcazole attenuates locomotor stimulation by cocaine confirms previous similar findings by other researchers. The IRP team established for the first time an additional potentially important effect of rimcazole-like compounds: In rats, a closely related compound called SH3-28 weakens the subjective sensations that distinguish the cocaine experience. Rats pretreated with SH3-28 lost some of their ability to tell the difference between injections of cocaine and injections of saline—plain salt water.

Pretreatment With Rimcazole Analog SH3-28 Decreases Subjective Effects of Cocaine in Rats

In this experiment, the researchers first taught rats they could obtain food by choosing the correct lever between two

options: To be rewarded, the animals needed to push one lever after receiving a cocaine injection and the other lever after receiving a saline injection. Once trained, rats pressed the cocaine-associated lever nearly 100% of the time after an injection of cocaine; however, when pretreated with 19 µmol/kg of SH3-28 prior to receiving cocaine, they pushed the correct lever only about 60% of the time. Should these results carry over to people, rimcazole or a rimcazole-like medication might be used in treatment of cocaine abuse. Recovering individuals who abused cocaine while taking the medication would learn that the drug did not produce the desired stimulant sensations, reducing their motivation for future use.



In rats conditioned to press a lever in response to cocaine injection, SH3-28 pretreatment reduced the accuracy of the animals' responses to cocaine.

"This area of research is still in the early stages of effectiveness testing, but if the present results prove reliable and can be extended to humans, it would appear that rimcazole and its analogs may have promise in further drug discovery efforts toward the treatment of cocaine abuse," says Dr. Frank Vocci, director of NIDA's Division of Pharmacotherapies and Medical Consequences of Drug Abuse.

The Mechanisms of Action

Rimcazole achieves its cocaine-suppressing effects at least in part by binding to and blocking the sigma receptor. This is a protein on the surface of some brain cells that receives chemical messages and relays them to the interior of the cells, stimulating or inhibiting some cellular activities. Compounds that block the sigma receptor inhibit cells from responding to dopamine, a chemical messenger that contributes to many of the addictive effects of cocaine as well as of other addictive drugs.

Rimcazole also binds to another protein on the cell surface, called the dopamine transporter. Most chemicals that attach here mimic the effects of cocaine, which itself is a potent dopamine transporter blocker. The IRP researchers are currently investigating whether rimcazole and rimcazole-like compounds produce an effect opposite to cocaine's because their sigma blockade overrides their dopamine transporter effect, or whether some more complicated interaction between the two comes into play. So far, the evidence seems to point to the latter possibility.

"Our findings suggest that the interaction of sigma receptor ligands and cocaine is complex and appreciably different from competitive antagonism—that is, rimcazole and its analogs do not appear to physically block cocaine from its binding site," says Dr. Katz. "It is possible that the effects of these compounds are due to the particular balance of dopamine transporter and sigma receptor actions they produce."

The IRP team's research constitutes the initial studies in a program of drug discovery. Future investigations will examine whether rimcazole and related compounds block other stimulant-induced effects of cocaine, attempt to isolate the exact nature of their effects at the sigma receptor and dopamine transporter, and—if the compounds continue to show promise—evaluate their safety and efficacy in animals and people.

Source

Katz, J.L., et al. Behavioral effects of rimcazole analogues alone and in combination with cocaine. *European Journal of Pharmacology* 468(2):109-119, 2003. [Abstract]

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