

Neuropeptide Promotes Drug-Seeking and Craving in Rats

Orexin emerges as a link in the chain of brain mechanisms regulating appetite for rewards.

BY LORI WHITTEN,
NIDA Notes Staff Writer

Orexin, a neuropeptide that stimulates eating and regulates wakefulness, also fosters animals' drug seeking and craving responses to drugs, according to two NIDA-funded studies. The research teams, led by Drs. Glenda Harris and Gary Aston-Jones at the University of Pennsylvania and Drs. Stephanie Borgland and Antonello Bonci at the University of California, San Francisco (UCSF), used different experimental procedures and studied different drugs. Their findings, however, point to the same conclusion: Augmenting orexin increases drug seeking, while blocking it has the opposite effect.

Orexin, also called hypocretin, is produced by neurons in the hypothalamus—a brain structure that regulates hunger, thirst, sleep, and other processes essential to survival. Scientists recently

discovered that people with narcolepsy lack orexin-producing neurons. The finding suggested an explanation for a striking observation made in the mid-1970s: People with narcolepsy rarely became addicted to the potent stimulants used to treat the disorder at the time. Perhaps, some scientists speculated, orexin contributes to the development of drug abuse.

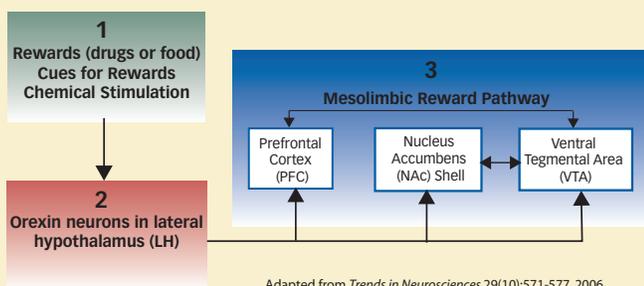
OREXIN AND DRUG SEEKING

An observation in animals by Drs. Harris and Aston-Jones also seemed to suggest a possible connection. They noted that lateral hypothalamus (LH) cells in the same area as orexin neurons were activated during drug seeking using a behavioral assay called conditioned place preference (CPP; for more on CPP, see "Animal Experiments in Addiction Science," *NIDA Notes* Vol. 20, No. 5). After repeated morphine injections in one chamber of a test cage and saline in the other, rats gravitate to the drug-

paired area in an effort to re-experience the opiate effects. The time they spend in the area—their morphine place preference—indicates how intensely the drug motivates drug seeking. When Drs. Harris and Aston-Jones determined that the LH neurons activated during drug seeking produce orexin, they conducted further experiments.

The Pennsylvania researchers first demonstrated that activa-

OREXIN STIMULATION DRIVES DRUG-SEEKING Exposure to cues associated with drugs activate the orexin neurons (red), which stimulates neurons in the brain's mesolimbic reward pathway (blue).



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NIDA Will Contribute to Obesity Research

The United States has a serious weight problem. Two-thirds of our adults are overweight or obese. The prevalence of overweight among our children has nearly tripled since 1970.

The consequences for the Nation's health and economy are grave. Obesity has been shown to decrease overall life expectancy and to increase the risk for cardiovascular disease, type 2 diabetes, and other chronic conditions. Annually, it costs an estimated \$117 billion or more in lost productivity and future earnings. To identify new strategies for prevention and treatment, the National Institutes of Health (NIH) has established an Agency-wide obesity task force and research plan (see obesityresearch.nih.gov).

NIDA's assignment on the 25-Institute task force represents a natural extension of the Institute's research agenda. Addiction and compulsive eating both involve impaired impulse control and distorted valuation of the rewards to be derived from a certain behavior—i.e., drug-taking or eating. The two conditions have roots in some of the same brain areas and circuits, including the hypothalamus, prefrontal cortex, and limbic system. The knowledge NIDA-funded scientists have developed of how those areas function normally and in the context of drug abuse undoubtedly will have great application to understanding the other compulsive behavior.

In this issue, we report an exemplary illustration of the neurobiological overlap between addiction and eating disorders: The hormone orexin appears to foster cravings for both food and drugs (see p. 1). Several other NIDA-supported studies are investigating compounds found to be effective in suppressing appetite and food intake. Some of the most promising produce their effects by blocking the brain's cannabinoid receptors, which are targeted by THC, the active ingredient in marijuana (cannabis), a drug with marked effects on appetite. Researchers hope to translate this knowledge into medications for weight control, drug abuse treatment, or both.

EFFORTS BY OTHER INSTITUTES IN THE COLLABORATION INCLUDE:

- A program, led by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), to foster a multidisciplinary approach to clinical obesity research in areas such as metabolism, endocrinology, nutrition, cardiovascular biology, digestive diseases, genetics, and behavioral sciences; and
- Projects to combat pediatric obesity in primary care, day care, schools, and other community settings by exploring effective methods to curb weight gain, including changes in food and lifestyle choices.

We hope that further research into the many factors that contribute to obesity—including neural systems that modulate appetite and eating behavior—will lead to the development of new therapies for obesity as well as addiction. ■

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Tracing NET

Researchers have developed and successfully tested a new tool for studying the neurobiology of depression, attention deficit hyperactivity disorder, and stimulant abuse.

Researchers will be able to use the tool, a new radiotracer, to map the location and circulation of a protein that plays a central role in those conditions: the norepinephrine transporter (NET). Drs. Yu-Shin Ding, Jean Logan, and colleagues at Brookhaven National Laboratory screened six molecules to see whether any of them did the things a radiotracer for NET must do: get into the brain, spread to regions of the brain with high concentrations of NET, bind selectively to the protein, and leave the brain in a reasonable amount of time. Of the six, (S,S)-[11C]methylreboxetine (MRB) was by far the best candidate, binding tightly and specifically to NET. The norepinephrine, dopamine, and serotonin transporters are

disrupted in depression, attention disorders, and stimulant abuse. Researchers previously developed radiotracers for the dopamine and serotonin transporters; the ability to image all three transporters in the brain will allow researchers to determine the role of each neurochemical in the disorders.

> *Journal of Neurochemistry* 94(2):337-351, 2005.



Controlling College Students' ADHD Symptoms May Protect Them Against Substance Abuse

In a survey of 334 students at a college in the Southeast, those who reported having experienced symptoms of attention deficit hyperactivity disorder (ADHD) during the past 6 months were three times as likely as those whose symptoms were controlled to say they had intensified their smoking, and six times as likely to report increased frequency of abuse of other drugs

(besides alcohol and marijuana) during the past year. Of the 76 respondents who said they had been prescribed ADHD medications at some point during their lives, one-quarter said they had abused their medication to get high, and 29 percent said they had given or sold it to someone else. Dr. Himanshu Upadhyaya and colleagues at the Medical University of South Carolina conducted the study. Appropriate treatment of ADHD may reduce college students' risk of drug abuse.

> *Journal of Child and Adolescent Psychopharmacology* 15(5):799-809, 2005



Exposure to Morphine During Early Adolescence Sensitizes Rats as Adults

A study conducted at Emory University School of Medicine indicates that exposure to morphine during adolescence may increase sensitivity to the drug during adulthood. Drs. Stephen

Holtzman and David White first established that both peri-adolescent (7 to 10 days before puberty onset) and adult male rats receiving 10 mg/kg/day of morphine for 1 day or 3 consecutive days exhibited similar increases in locomotor activity relative to age-matched rats receiving only saline. When the researchers reexposed the animals to morphine 5 weeks later, significant age- and exposure-related differences emerged. All of the rats were more active, but a relatively small dose of morphine (0.3 mg/kg) triggered significantly more activity in the rats that previously were exposed to morphine for 3 days as periadolescents, relative to their 1-day and unexposed counterparts. By contrast, rats previously exposed for 3 days as adults required 10 times that dose to exhibit more activity than those in the 1-day and unexposed groups. The findings suggest that, during adolescence, even a relatively low level of exposure to morphine can have profound, long-lasting effects.

> *European Journal of Pharmacology* 528(1-3):119-123, 2005.

NIDA's Division of Clinical Neuroscience and Behavioral Research

NIDA's Newest Division Mines Clinical Applications From Basic Research

BY DEBRA P. DAVIS,
NIDA Notes Staff Writer

NIDA's Division of Clinical Neuroscience and Behavioral Research (DCNBR) identifies, validates, and explores the clinical implications of basic science discoveries. Much of the Division's work consists of replicating results obtained in laboratory and animal studies in human subjects. A DCNBR project typically culminates in one of two outcomes: carrying a new discovery forward for development into actual interventions or referring it back to basic scientists for further investigation.

"We are uniquely positioned to uncover the factors in humans—neurobiologic, genetic, social-behavioral—that help explain the development and effects of drug abuse," says Director Dr. Joseph Frascella. "Being positioned between NIDA's basic research division and other more applied programs, our research programs inform basic science and promote the development and implementation of new medications and behavioral treatments across NIDA."

For example, building on basic research that linked nicotine acetylcholine receptors to the regulation of attention, DCNBR-sponsored researcher David Gilbert and colleagues at Southern

Illinois University demonstrated that nicotine exposure and smoking cessation both influence the ability to pay attention. Now, NIDA's Division of Pharmacotherapies and Medical Consequences of Drug Abuse is exploring the clinical impact of these observations. They are testing whether bupropion and nicotine patches affect attention during smoking cessation, and whether such effects correlate with success in quitting.

In another DCNBR-supported study, Dr. Robert Risinger and colleagues at the Medical College of Wisconsin documented a pattern of fluctuating activation of the mesolimbic reward system as six cocaine-abusing men transitioned through cycles of cocaine craving, self-administration, and highs. The results, obtained with functional magnetic resonance imaging (fMRI), confirmed previous research linking those brain areas to drug self-administration in animals, and extended them by correlating them with drug abusers' subjective feelings and responses. (see "Cocaine Craving Activates Brain Reward Structures; Cocaine 'High' Dampens Them," *NIDA Notes*, Vol. 21, No. 2).

Neuroimaging studies comprise one-third of the Division's research portfolio. "Brain imaging has pushed drug abuse research in the last 15 to 20 years—allowing us to directly observe neural activity

of awake and functioning people and obtain specific measures on how drugs affect the brain," Dr. Frascella says. Imaging studies indicate that the human brain undergoes major changes as a consequence of drug exposure and that the adolescent brain may be particularly pliant, "which may help explain why adolescence is the period when most new cases of drug addiction occur," Dr. Frascella adds. "Thus, we've become increasingly committed to using brain imaging to get a sharper picture of how the brain changes with development and in response to active and passive exposure to drugs of abuse."

THREE BRANCHES OF CLINICAL RESEARCH

DCNBR, formed in May 2004, has three Branches:

Clinical Neuroscience: Under Dr. Steven Grant's leadership, the Clinical Neuroscience Branch focuses on how drug abuse affects the human central nervous system, including brain changes during different stages and states of abuse, such as addiction, withdrawal, abstinence, craving, and relapse. It also studies:

- The factors that make some individuals more vulnerable and others more resilient to drug abuse and addiction;
- The impact of drugs on learning, memory, judgment, and decision-making;
- Co-occurrence of drug addiction with other mental disorders;
- Neurobiological changes that result from behavioral and pharmacological treatments for drug abuse; and
- Interventions to ameliorate the negative health consequences of drugs, whether self-administered or incurred prenatally.

As an example of a recent Branch achievement, Dr. Grant points to a study by Dr. Martin Paulus and colleagues at the University of California, San Diego.

The researchers performed fMRI while 46 men who had been abstinent from methamphetamine for about a month took a decisionmaking test. The researchers found that patterns of activation in certain brain regions predicted with high accuracy which men would and would not relapse 1 year later (see “Brain Activity Patterns Signal Risk of Relapse to Methamphetamine,” *NIDA Notes*, Vol. 20, No. 5). “This use of fMRI, if replicated, could be adapted for treatment and preventive interventions,” Dr. Frascella says.

Behavioral and Brain Development:

Led by Dr. Vincent Smeriglio, this Branch examines drug exposure and abuse, and their health and social consequences, through the course of human development—from the prenatal period through childhood, adolescence, and young adulthood. Research focuses on:

- The effects of drugs on behavioral and brain development;
- The role of genetic, neurobiological, and environmental factors in youths’ vulnerability to drug abuse and addiction; and
- The developmental effects of co-occurring exposure to drugs and infectious diseases as well as the impact of drug abuse on youths with mental illness.

One theme of the Branch’s work is research to tailor drug abuse treatments to individual needs. As a recent example, a Branch-supported study by Dr. Leslie Jacobsen and colleagues at Yale University School of Medicine found that adolescent smokers experienced greater memory impairment during nicotine withdrawal if their mothers had smoked while pregnant. The study also produced fMRI evidence implicating particular brain regions in the deficits, which may be useful diagnostically as well as therapeutically.

Behavioral and Integrative Treatment: This Branch, under Dr. Lisa

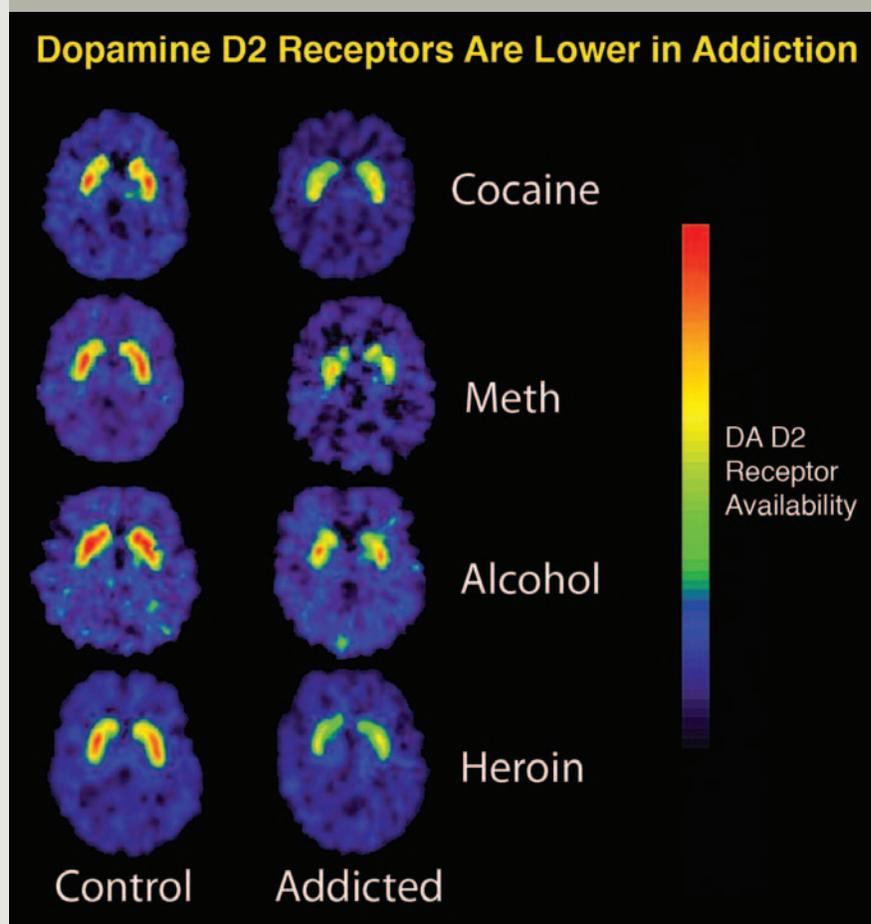
Onken’s direction, aims to develop new and improved treatments for drug abuse and addiction, including behavioral, combined behavioral-pharmacological, and complementary and alternative treatments. The Branch designs new interventions, tests them for efficacy, and evaluates strategies to improve treatment engagement, adherence, and retention—key factors in success. The Branch also supports research that prepares treatments for use in community settings, where cost, training, and fit with existing services can be constraints.

“Behavioral therapies that work well in a research setting cannot necessarily be taken into the community. Often-

times, they are too complicated and expensive, and require extensive training for clinicians,” Dr. Frascella notes. One way of developing a community-friendly treatment is to identify the active ingredients of effective treatments, explains Dr. Onken. “In this way, we may be able to create more streamlined treatments that retain their potency. Another novel approach to making a treatment more community-friendly is to use computers to help counselors deliver treatment. For example, Dr. Kathleen Carroll at Yale University is testing the efficacy of computer-assisted cognitive-behavioral therapy in preventing cocaine relapse.” ■

BRAIN IMAGING IS A KEY TOOL IN STUDIES SPONSORED BY THE DIVISION OF CLINICAL NEUROSCIENCE AND BEHAVIORAL RESEARCH

The images below—used in a recent Division presentation—show that repeated exposure to drugs depletes the brain’s dopamine receptors, which are critical for one’s ability to experience pleasure and reward.



OREXIN

[Continued from page 1]

tion of orexin neurons in the LH was tightly coupled with rats' place preferences for morphine, cocaine, and sweet food. Next, they gave a different group of rats morphine for 3 days to establish place preference, then stopped the drug and injected some rats with a compound (SB334867) that prevents orexin from interacting with brain cells. Following treatment with SB334867, rats spent 58 percent less time in the morphine-associated cage area—indicating a halving of their drug seeking. Rats given inert vehicle showed no significant change in drug seeking.

The investigators also tested orexin's impact on the tendency of a new group of rats to revert to drug seeking after CPP waned following extended testing without drug administration (extinction). In contrast to the first experiment, this time the investigators injected a compound (rat pancreatic polypeptide, rPP) that stimulates orexin neurons into the LH of some animals. These rats quickly resumed CPP—indicated by the difference in time spent in the morphine versus saline chambers—as marked as that of another group that received a morphine priming injection (353 seconds and 424 seconds for rPP and morphine, respectively). Rats that received a vehicle injection did not renew morphine CPP.

To establish that stimulation of orexin neurons by rPP, and not some other unidentified factor, was responsible for the effects in their second experiment, the investigators repeated the procedure. This time they blocked the extinction of orexin by giving the rats SB334867 prior to rPP. These rats did not resume CPP. Finally, the researchers infused orexin directly into rats' ventral tegmental area (VTA), the origin of the dopamine-rich reward pathway, and observed a resumption of drug-seeking behavior.

DRUGS MAY USURP FEEDING SYSTEM

The results, although in animals, suggest that orexin promotes drug abusers' desire

for drugs and their risk for relapse. "It makes sense, anatomically and physiologically, that orexin might play a role in reward-seeking and craving," says Dr. Harris, now at the Centre de Regulacio Genomica in Spain. "Neurons in this part of the brain stimulate eating; intense cravings for food and water originate here. Our findings suggest that orexin from the lateral hypothalamus affects the reward pathway. Perhaps drugs take over the brain system for feeding and craving just as they usurp neural systems for reward."

"These behavioral findings extend the team's important anatomical work differentiating two populations of orexin-producing neurons in the hypothalamus. One population, located in the lateral hypothalamus, is involved in feeding, reward, and drug seeking, while the other regulates sleep and arousal," says Dr. Susan Volman of NIDA's Division of Basic Neuroscience and Behavioral Research. "The findings identify new neural pathways involved in drug abuse, craving, and relapse, and may ultimately help scientists find more effective therapies."

ROLE IN COCAINE CRAVING?

Drs. Borgland and Bonci and colleagues at the UCSF Ernest Gallo Clinic and Research Center demonstrated orexin effects on cocaine-related behaviors remarkably consistent with those the Pennsylvania team showed with respect to drug-related behaviors. They also provided evidence that orexin produces these effects at least in part by altering neurons in the VTA. The UCSF team used behavioral sensitization to evaluate orexin's impact on rats' responses to cocaine. Scientists generally think animals' behavioral sensitization—increased locomotor activity following repeated exposure to a drug—reflects drug-induced neural changes and corresponds to human craving for the drug. In the UCSF experiment, rats pretreated with an orexin blocker displayed only half as much increase in locomotor activity (138 percent) following five daily cocaine infusions (15 mg/kg) as rats pretreated with an inert vehicle (257 percent).

To explore the cellular bases for their behavioral observations, the UCSF group measured orexin's effects on the electrophysiological properties of dopamine-producing cells in brain slices removed from the VTA of rats. The results showed that orexin increased the number of receptors for neural excitation on the surfaces of these cells. Such strengthening of intercellular connections occurs during learning. Scientists believe it may foster the development of drug craving. When the researchers pretreated the rats with an orexin blocker, cocaine lost its ability to alter dopamine-producing cells in the VTA, suggesting that orexin may be necessary for cocaine-induced neuroplasticity and its behavioral consequences.

"Our findings point to a key role for orexin in the neural changes in the reward pathway that underlie craving and relapse," says Dr. Borgland. "The physiological alterations we observed likely influence those cells' dopamine release, perhaps affecting the activity of the reward pathway in a way that increases the likelihood of relapse. One implication of our findings is that addiction medication development efforts might do well to target orexin receptors," she says.

The work of the Pennsylvania and UCSF teams points to orexin involvement in reward-seeking in general. Researchers studying the effects of orexin-blocking compounds in animal models of alcoholism and obesity have reported preliminary but promising findings. Both teams are currently determining whether giving such compounds to animals reduces self-administration of cocaine, say Dr. Bonci and Dr. Aston-Jones, the latter now at the Medical University of South Carolina. ■

SOURCES

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Endorphin Derivative Inhibits Reward From Morphine and Nicotine in Rats

Recent studies suggest therapeutic potential for glycyl-glutamine in opiate and nicotine addiction.

BY SARAH TEAGLE,
NIDA Notes Contributing Writer

A naturally occurring brain chemical has shown early promise as a treatment for addiction. NIDA-funded researcher Dr. William Millington and colleagues at Albany College of Pharmacy demonstrated that glycyl-glutamine (Gly-Gln), a product of the conversion of one form of beta endorphin to another, reduces the rewarding effects of morphine and nicotine and the severity of withdrawal from these drugs in rats.

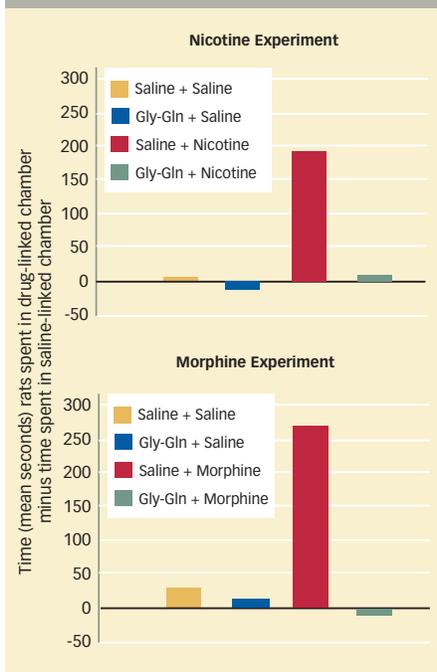
The researchers utilized an animal model called conditioned place preference (CPP; for more on CPP, see “Animal Experiments in Addiction Science,” *NIDA Notes*, Vol. 20, No. 5). When pretreated with Gly-Gln, rats stopped preferring a cage in which they received morphine infusions over another in which they received a physiologically inert substance. The investigators concluded that Gly-Gln completely blocked the brain-rewarding effects of morphine. They used the same experimental technique to demonstrate that Gly-Gln pretreatment also blocks the rewarding effects of nicotine in rats.

Some chemicals that block the rewarding effects of drugs of abuse also take away subjects’ pleasure in normal healthy activities. Again using CPP, Dr. Millington’s team demonstrated that Gly-Gln does not have this drawback, at least in regard to one food—a sweetened cereal—that rats enjoy.

Subsequently, Dr. Millington and his coinvestigators examined Gly-Gln’s effect on withdrawal from morphine. They induced

GLY-GLN BLOCKS THE REWARDING EFFECTS OF NICOTINE AND MORPHINE

Rats trained to associate nicotine or morphine infusions with a particular chamber showed a clear preference for the drug-linked chamber when later allowed to roam freely between it and a second chamber. Rats that were pretreated with Gly-Gln prior to receiving nicotine or morphine divided their time roughly equally between drug-linked and saline-linked chambers.



morphine dependence in the rats, injected some with Gly-Gln 72 hours later, and then administered the opioid antagonist naloxone to induce withdrawal. Across all measures, the Gly-Gln pretreated rats exhibited significantly fewer behavioral and physiological signs of withdrawal than the others. The researchers observed a similar effect when they induced withdrawal from nicotine with the agent mecamylamine.

SAFE ANALGESIA, TOO?

In separate studies, Dr. Millington and colleagues found evidence that Gly-Gln has potential for improving pain treatment by slowing the development of morphine tolerance. The investigators treated rats with morphine twice daily for 7 days and, each day, measured the rats’ reaction to pain with tail-flick latency tests. They observed that the pain-relieving effects of morphine declined 20 percent by the second day, an indication that tolerance had developed rapidly. However, rats pretreated with Gly-Gln did not begin showing evidence of morphine tolerance until the fourth day of treatment. Their level of pain relief had dropped to 75 percent of the maximum by day 4, compared with 39 percent for rats that were not pretreated.

These findings offer new hope for making pain treatment more effective, but without adding to the problems of painkiller abuse and prescription drug diversion. Dr. David Thomas of NIDA’s Division of Basic Neuroscience and Behavioral Research explains, “It is like a tug-of-war; we want better pain treatment, but we do not want more addiction. If we can find a medication that improves the management of chronic pain without causing addiction or the negative physical side effects, then it really is a win-win situation. Gly-Gln may give us that.”

“We need to learn much more about Gly-Gln’s pharmacology before we can develop it into a drug that is useful clinically,” says Dr. Thomas. “The profile of its effects brought out by Dr. Millington’s work is striking. It makes a strong argument for taking the research to the next step: understanding its mechanism of action in the brain. In our world of preclinical animal research, we really could not ask for better findings. So far, everything we know about Gly-Gln is promising.”

SOURCES

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Goktalay, G., et al. Glycyl-glutamine inhibits nicotine conditioned place preference and withdrawal. *European Journal of Pharmacology* 530(1-2):95-102, 2005.

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

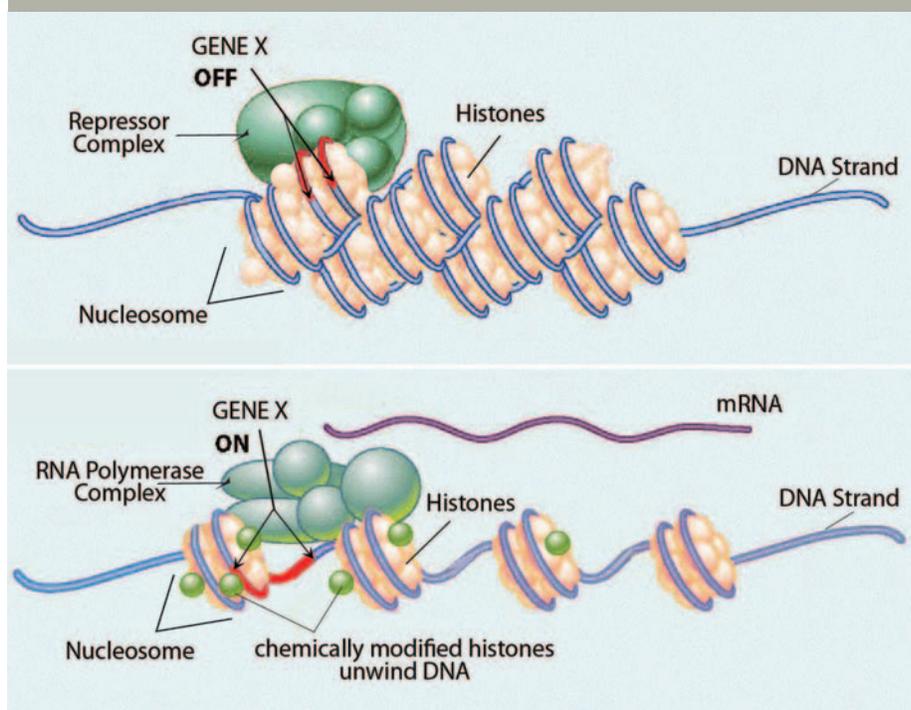
Cocaine produces the long-term brain changes that underlie addiction in part by activating certain genes. Dr. Eric Nestler and colleagues at the University of Texas Southwestern Medical Center and Harvard Medical School have shown that the drug achieves this activation at least in part through a process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

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, FosB, BDNF, and Cdk5, 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 FosB correlates with cocaine craving and drug self-administration in animals, and may contribute to long-lasting 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

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.



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 (H3) and histone 4 (H4). 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 H4 than that in the control animals, and phosphoacetylated H3 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 H4 in chromatin linked to the *FosB* gene, but the levels returned to baseline within 3 hours. Repeated cocaine did not induce H4 acetylation in *FosB* gene-associated chromatin, but did cause H3 acetylation. The researchers say that the switch from H4 acetylation after a single cocaine exposure to H3 acetylation after chronic exposure may mark a turning point in developing addiction.

"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 H3 acetylation and *FosB*'s activation and subsequent triggering of *FosB* 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 transi-

tion 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 H3 acetylation of both. Once initiated, the effects were long-lasting. The quantities of modified H3 in *BDNF* gene-associated chromatin in exposed animals increased from 3-fold of those of saline-treated animals at day 1 to 14-fold at day 7. Acetylated H3 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 *FosB* protein selectively activated *Cdk5*—the only gene examined in the study that was turned on in this way. This finding suggests that *FosB* 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

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.

GENE EXPERIMENT

[Continued from page 9]

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 cue-induced 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,” *NIDA Notes*, Vol. 20, No. 5). The researchers hypothesized that augmenting or preventing histone modifications during drug administration sessions would increase or decrease CPP, respectively.

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-associ-

ated 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 self-administered 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.

COCAINE TURNS ON GENES BY ALTERING CHROMOSOMAL PROTEINS The researchers found chemical modifications to histone 3 (H3) and histone 4 (H4)—major proteins that form the structure of chromosomes—at areas linked with four genes. Acetylation of H3 and H4 and phosphoacetylation of H3 alter the proteins’ chemical structure, facilitating gene activation.

Experiment	Gene	Results					
		30 min.	1 hr.	90 min.	3 hr.	1 day	7 days
<i>Acute Cocaine</i> Rats received a single injection, either saline or cocaine (20 mg/kg).	<i>cFos</i>	Acetylated H4 Phosphoacetylated H3		Normal Histones	Normal Histones	----	
	<i>FosB</i>	Acetylated H4					
	<i>BDNF</i>	No change			No change		
	<i>Cdk5</i>	No change			No change		
<i>Chronic Cocaine</i> Rats received an injection of either saline or cocaine (20 mg/kg) daily for 7 days.	<i>cFos</i>	No change			No change		
	<i>FosB</i>	Acetylated H3	Normal Histones				
	<i>BDNF</i>	Acetylated H3				Normal Histones	
	<i>Cdk5</i>	Acetylated H3				Normal Histones	
<i>Cocaine Withdrawal</i> Rats received an injection of either saline or cocaine (20 mg/kg) daily for 7 days and did not receive the drug again.	<i>cFos</i>	Effects too transient to examine					
	<i>FosB</i>	Effects too transient to examine					
	<i>BDNF</i>	Acetylated H3: Levels rise to 3-fold those associated with saline injection after 1 day, and to 14-fold after 7 days.					
	<i>Cdk5</i>	Acetylated H3: Levels rise to 3-fold those associated with saline injection after 1 day, and return to normal after 7 days.				Normal Histones	

SOURCE

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

Impacts of Drugs on Neurotransmission

The defining features of drug intoxication and addiction can be traced to disruptions in cell-to-cell signaling.

BY CARL SHERMAN, *NIDA Notes Contributing Writer*

Drugs of abuse alter the way people think, feel, and behave by disrupting neurotransmission, the process of communication between brain cells. Over the past few decades, studies have established that drug dependence and addiction are features of an organic brain disease caused by drugs' cumulative impacts on neurotransmission. Scientists continue to build on this essential understanding with experiments to further elucidate the physiological bases for drug abuse vulnerability as well as the full dimensions and progression of the disease. The findings provide powerful leads to new medications and behavioral treatments.

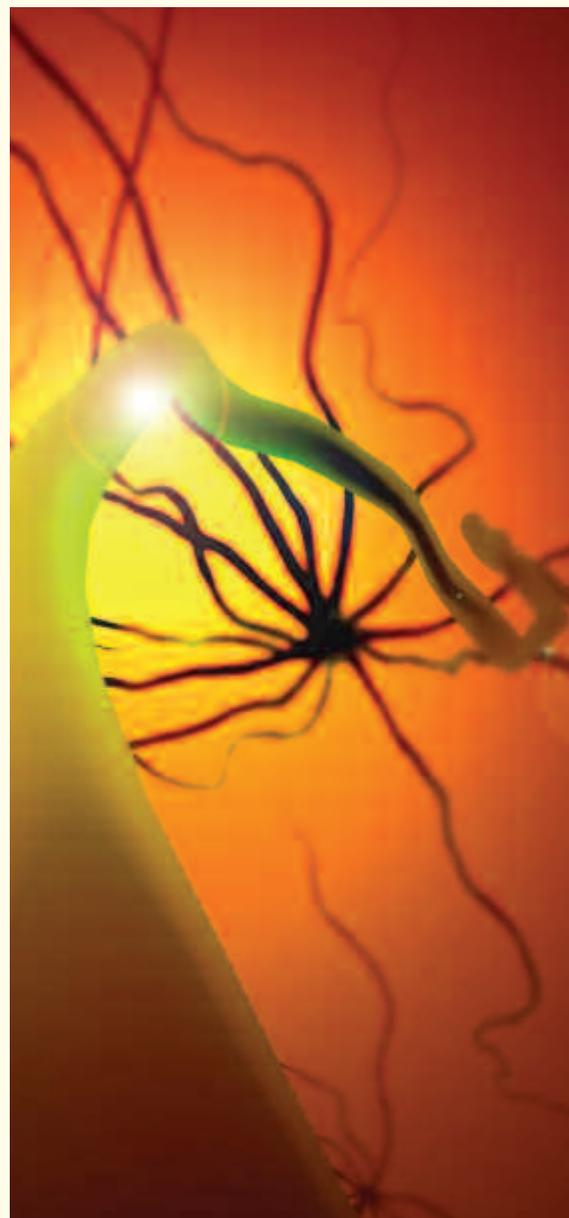
This second article in our *NIDA Notes Reference Series* discusses the central importance of studying drugs' effects on neurotransmission and describes some of the most common experimental methods used in this research. As with other articles in the series, we provide illustrative references from articles published in *NIDA Notes*.

WHAT IS NEUROTRANSMISSION?

A person reads. The words on the page enter the brain through the eyes and are transformed into information that is relayed, from cell to cell, to regions that process visual input and attach meaning and memory. When inside cells, the information takes the form of an electrical signal. To cross the tiny intercellular gap that separates one cell from the next, the information takes the form of a chemical signal. The specialized chemicals that carry the signals across the intercellular gaps, or synapses, are called neurotransmitters.

The ebb and flow of neurotransmitters—neurotransmission—is thus an essential feature of the brain's response to experience and the environment. To grasp the basic idea of neurotransmission, compare the brain to a computer. A computer consists of basic units (semiconductors) that are organized into circuits; it processes information by relaying electric current from unit to unit; the amount of current and its route through the circuitry determine the final output. The brain's corresponding basic units are the neurons—100 billion of them; the brain relays information from neuron to neuron using electricity and neurotransmitters; the volume of these signals and their routes through the organ determine what we perceive, think, feel, and do.

Of course, the brain, a living organ, is much more complex and capable than any machine. Brain cells respond with greater versatility to more types of input than any semiconductor; they also can change, grow, and reconfigure their own circuits.



THE BASIC RESEARCH QUESTIONS

Neuroscientists seeking to understand why a drug is abused and the consequences of that abuse focus on two issues:

- Which neurotransmitter or neurotransmitters does it affect?
- How does it alter neurotransmission?

Which Neurotransmitter or Neurotransmitters Does the Drug Affect?

A person’s experiences when abusing a drug reflect the functional roles of the particular neurotransmitter whose activity it disrupts. Each individual neuron manufactures one or more neurotransmitters: dopamine, serotonin, acetylcholine, or any one of a dozen others that scientists have discovered to date. Each neurotransmitter is associated with particular effects depending on its distribution among the brain’s various functional areas. Dopamine, for example, is highly concentrated in regions that regulate motivation and feelings of reward, accounting for its importance in compulsive behaviors such as drug abuse. A neurotransmitter’s impact also depends on whether it stimulates or dampens activity in its target neurons.

Some drugs primarily disrupt one neurotransmitter or class of neurotransmitters. For example, opioid drug abusers experience changes that are similar to—but more pronounced than—those that accompany normal fluctuations in the brain’s natural opioid-like neurotransmitters, endorphin and enkephalin: increased analgesia, decreased alertness, and slowed respiration (see table). Other drugs interact with more than one type of neurotransmitter. Cocaine, for example, attaches to structures that regulate dopamine, thereby producing euphoria; however, cocaine also produces changes in norepinephrine and glutamate, which are the sources of its stimulant effects.

Because a neurotransmitter often stimulates or inhibits a cell that produces a different neurotransmitter, a drug that alters one can have secondary impacts on another. In fact, the key effect that all abused drugs appear to have in common—a dramatic increase in dopamine signaling in the nucleus accumbens (NAc), leading to euphoria and a desire to repeat the experience—is in many cases an indirect one. For example, nicotine stimulates dopamine-releasing cells directly by stimulating their acetylcholine receptors, and also indirectly by triggering higher levels of glutamate, a neurotransmitter that acts as an accelerator for neuron activity throughout the brain.¹

How Does the Drug Alter Neurotransmission?

Neurotransmission is a cyclic process that transpires in several steps utilizing specialized components of the sending and receiving cells (see “Getting the Message Across”). Identifying the precise step that a drug disrupts, and how, provides crucial insight into

Neurotransmitter	Distribution in the Central Nervous System	Functions Affected	Drugs That Affect It
Dopamine	Midbrain, Ventral tegmental area (VTA), Cerebral cortex, Hypothalamus	Pleasure and reward, Movement, Attention, Memory	Cocaine, Methamphetamine, Amphetamine. In addition, virtually all drugs of abuse directly or indirectly augment dopamine in the reward pathway
Serotonin	Midbrain, VTA, Cerebral cortex, Hypothalamus	Mood, Sleep, Sexual desire, Appetite	MDMA (ecstasy), LSD, Cocaine
Norepinephrine	Midbrain, VTA, Cerebral cortex, Hypothalamus	Sensory processing, Movement, Sleep, Mood, Memory, Anxiety	Cocaine, Methamphetamine, Amphetamine
Endogenous opioids (endorphin and enkephalin)	Widely distributed in brain but regions vary in type of receptors, Spinal cord	Analgesia, Sedation, Rate of bodily functions, Mood	Heroin, Morphine, Prescription painkillers (Oxycodone)
Acetylcholine	Hippocampus, Cerebral cortex, Thalamus, Basal ganglia, Cerebellum	Memory, Arousal, Attention, Mood	Nicotine
Endogenous cannabinoids (anandamide)	Cerebral cortex, Hippocampus, Thalamus, Basal ganglia	Movement, Cognition and memory	Marijuana
Glutamate	Widely distributed in brain	Neuron activity (increased rate), Learning, Cognition, Memory	Ketamine, Phencyclidine, Alcohol
Gamma-aminobutyric acid (GABA)	Widely distributed in brain	Neuron activity (slowed), Anxiety, Memory, Anesthesia	Sedatives, Tranquilizers, Alcohol

its impact on abusers and is key to identifying medical and behavioral interventions to inhibit, counter, or reverse the disruption.

Some drugs mimic neurotransmitters. Opioid drugs such as heroin and OxyContin, for example, chemically resemble the brain’s natural opioids sufficiently to engage and stimulate their specialized receptors. Since heroin stimulates many more receptors than the brain uses in the normal cycle of endorphin and enkephalin release and uptake, the result is a massive amplification of opioid activity. Marijuana and hashish mimic cannabinoid neurotransmitters, the most important of which is anandamide. Nicotine attaches to receptors for acetylcholine, the neurotransmitter for the cholinergic system.

Some drugs alter neurotransmission by interacting with molecular components of the sending and receiving process other than receptors. Cocaine, for example, attaches to the dopamine trans-

porter, the molecular conduit that draws free-floating dopamine out of the synapse and back into the sending cell. As long as cocaine occupies the transporter, dopamine cannot reenter the cell by this route. It builds up in the synapse, stimulating receiving cell receptors more copiously and producing much greater dopamine impact on the receiving cells than occurs naturally. “Cocaine’s Dopamine Connections” enumerates some of cocaine’s interactions with the mechanisms of dopamine signaling, and how they motivate abuse and contribute to dependence and addiction.

Finally, some drugs alter neurotransmission by means other than increasing or decreasing the quantity of receptors stimulated. Benzodiazepines, such as diazepam or lorazepam, enhance receiving cells’ responses when the neurotransmitter gamma-aminobutyric acid (GABA) attaches to their receptors. Benzodiazepines’ relaxation effects result from this increased sensitivity to GABA’s inhibitory impact on cellular activity.

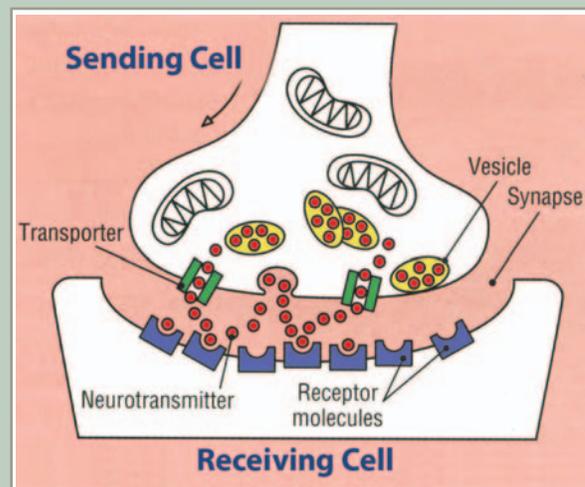
What Changes Occur With Chronic Drug Abuse?

During the early phase of an individual’s drug experimentation, neurotransmission normalizes as intoxication wears off and the substance leaves the brain. Eventually, however, drugs wreak changes in cellular structure and function that lead to long-lasting or permanent neurotransmission abnormalities. These alterations underlie drug tolerance, addiction, withdrawal, and other persistent consequences.

Some longer term changes begin as adjustments to compensate for drug-induced increases in neurotransmitter signaling intensities. For example, drug tolerance typically develops because sending cells reduce the amount of neurotransmitter they produce and release, or receiving cells withdraw receptors or otherwise dampen their responsiveness. Scientists have shown, for example, that cells withdraw opioid receptors into their interiors (where they cannot be stimulated) when exposed to some opioid drugs; when exposed to morphine, however, cells appear instead to make internal adjustments that produce the same effect—reduced responsiveness to opiate drugs and natural opioids.² Over time, this and related changes recalibrate the brain’s responsiveness to opioid stimulation downward to a level where the organ needs the extra stimulation of the drug to function normally; without the drug, withdrawal occurs.

The drug-related mechanisms producing cumulative changes in neurotransmission sometimes are genetic in nature. While a drug cannot change a person’s genes, drugs can prod some genes to increase their production of proteins, leading to changes in cell function or even actual reshaping of the physical structure of cells. For example, in rats, cocaine and amphetamine stimulate genes that produce the proteins used to build dendrites, branch-like cell structures that contain neurotransmitter receptors.³ Brains normally sprout new dendrites as they register new learning; the accelerated dendrite formation stimulants induce may partially account for these drugs’ unusual hold on an abuser’s attention.

Getting the Message Across



The task in neurotransmission is to convey a signal from a sending cell to a receiving cell across an open space known as a *synapse*. All brain cells accomplish this in approximately the same way.

The sending cell manufactures neurotransmitter molecules and stores them in packets called *vesicles*. When stimulated appropriately, the cell generates an electric signal and causes some vesicles to migrate to the cell membrane, merge with it, open up, and release their contents into the synapse. Some molecules drift across the synapse and link up, lock-and-key fashion, with molecules called *receptors* on the surface of the receiving cell. Receptors bridge the receiving cell’s membrane; they have one facet on the outside and one on the inside of the cell. When the neurotransmitter links up with the exterior facet, the interior facet precipitates an electrical response in the cell membrane or inside the cell. The result may be increased production of some cell product or—often—a repeat of the process just described, so that the message gets relayed in turn to the next cell in the circuit.

At this point, cell-to-cell communication is complete. The neurotransmitter molecules drop off the receptors. Loose again in the synapse, they meet three fates:

- Some attach to another receptor;
- Some encounter an *enzyme*, a chemical that breaks them apart; and
- Some reenter the sending cell via a special pathway through the axon membrane, called a *transporter*. Once back inside the cell, they are available for re-release in future neurotransmission episodes.

Normally, when drugs are not present, the cycle of release, breakup, and cell re-entry maintains the amount of neurotransmitter in the synapse, and hence neurotransmission, within certain limits. In most cases, when an abused drug enters the brain, it causes neurotransmission to increase or decrease dramatically beyond these limits.

Some drugs are toxic to nerve cells, and the effect accumulates with repeated exposures. For example, the club drug methylenedioxymethamphetamine (MDMA, ecstasy) damages axons that release serotonin; the result is disruption of serotonin neurotransmission that likely underlies the long-lasting memory problems experienced by abusers.⁴ Similarly, methamphetamine, over time, damages enough dopamine-sending cells to cause significant defects in thinking and motor skills; with abstinence, dopamine function can partially recover, but it is unclear whether cognitive and motor capabilities come back as well.⁵

EXPERIMENTAL METHODS

To determine whether or how a drug affects a particular neurotransmitter, researchers typically will compare individuals who have a history of drug exposure with others who do not. If researchers are investigating links between a drug's impact on neurotransmission and a drug-related behavior or symptom, they may compare individuals who exhibit the behavior or symptom with others who do not. The subjects in these experiments may be animals or people. In the case of animals, drug exposure often takes place under laboratory conditions designed to mimic human drug consumption. Studies can be divided into those in which measurements are made in living animals or people and those in which animal brain tissue is removed and examined.

Brain Tissue Assays

With removed tissue, scientists may perform chemical assays to quantify the presence of a neurotransmitter, receptor, or other structure of interest. In a recent experiment, scientists assayed brain tissue from 35-day-old rat pups and found that those that had been exposed to nicotine *in utero* had fewer nicotine receptors in the reward system than unexposed rats.⁶

A second experimental method using removed brain tissue—*in vitro*, literally, *in glass*, a historical term referring to the containers for the tissue and solution—enables researchers to view a drug's effects on neurotransmission in action. Scientists place the tissue in a laboratory solution of nutrients that enables the cells to continue to carry out some of their living functions. The researchers may then, for example, add the drug being investigated to the solution and monitor whether the cells respond by increasing their release of neurotransmitters. Alternatively, they may measure cell membrane or electrical properties that stimulate or inhibit the release of neurotransmitters.

In both *in vitro* experiments and in living animals, the techniques for measuring neurotransmitter quantities and fluctuations include microdialysis and fast-scan cyclic voltammetry (FSCV). Microdialysis involves taking a series of samples of the intercellular fluid containing the neurotransmitter through a microscopic tube inserted into the tissue or living brain. FSCV, recently developed by NIDA-funded scientists, monitors neurotransmitter fluctua-

tions at tenth-of-a-second intervals by measuring electrical changes related to neurotransmitter concentrations.⁷

Live Studies

Studies with living animals or people are essential for tying drugs' effects on neurotransmitters to behaviors or symptoms. A common design for experiments with either animals or people is to give study subjects a chemical that has a known effect on a particular neurotransmitter, and then observe the impact on their behavior. Typically, the chemical is either an *agonist* (promoter) or *antagonist* (blocker) of signaling by the neurotransmitter.

In a recent experiment, for example, a research team administered a glutamate agonist to rats and showed that the resulting increased levels of the neurotransmitter correlated with a reduction in the animals' cocaine seeking.⁸ Another team using the same strategy implicated glutamate in nicotine withdrawal.⁹ Such studies are a staple of testing compounds to identify medication classes with potential for treating abuse or addiction.

Researchers also genetically alter animals to have special characteristics, such as producing less or more than the normal amounts of a particular neurotransmitter, or lacking receptors for a neurotransmitter. Researchers expose such animals to a drug and observe whether the animals' display of some particular drug-related behavior—for example, pacing restlessly after being given a stimulant—increases or decreases.

Brain Scans

Brain imaging techniques enable neuroscientists to directly assess neurotransmission in people and living animals. With positron emission tomography (PET), researchers can compare groups of drug-abusing and nonabusing individuals, quantifying differences in their levels of a particular neurotransmitter molecule (e.g., dopamine) or neurotransmission component (e.g., a receptor or transporter). With PET, researchers also can correlate a drug's transit through the brain with fluctuations in a target neurotransmitter. They can elicit a drug-related behavior or symptom (e.g., craving) and relate neurotransmitter fluctuations to the rise and fall in its intensity.

One recent PET study, for example, showed that smokers have less of the neurotransmitter-degrading enzyme monoamine oxidase-B (MAO-B) throughout their bodies than nonsmokers.¹⁰ The relative deficit of MAO-B may help explain why smokers are at higher risk for hypertension and other chronic diseases.

Researchers use both PET and functional magnetic resonance imaging (fMRI) to monitor metabolic activity in selected regions of the brain. Because each neurotransmitter has a unique distribution among the regions of the brain, information on locations of heightened or decreased activity provides clues to which neurotransmitter is affected under the conditions of the study.

Cocaine's Dopamine Connections

Research on cocaine illustrates that many dimensions may be involved in a single drug's interaction with the activity of a single neurotransmitter. Studies show that cocaine alters dopamine neurotransmission with effects on:

Reward

- Cocaine causes the pleasurable feelings that motivate drug abuse by raising dopamine concentrations in the synapses of the reward system.^a
- Besides keeping dopamine in the synapses by blocking the transporters, cocaine can indirectly promote release of additional dopamine into the synapses by mobilizing a supply that the sending cells normally hold in reserve.^b
- Cocaine's yield of pleasurable feelings arises largely through the activity of one particular set of dopamine receptors, called D3 receptors.^c

Addiction

- Some studies indicate that the transition from casual cocaine abuse to addiction begins with the abuser's very first doses. For example, a single exposure to cocaine causes some cells in the brain's reward system to increase their responsiveness to subsequent stimulations.^d
- In living animals with minimal exposure to cocaine, the drug alters the dopamine responsiveness for at least a week.^e
- After chronic cocaine abuse dopamine ticks up in the reward system when the abuser encounters a cue associated with the drug.^f
- Brains normally sprout new neurotransmitter receiving structures in the process of turning new experience into learning. Cocaine accelerates this process, which may help account for the drug's unusual hold on an addicted individual's attention.^g

Vulnerability to Abuse

- A young person's marked taste for novelty may be an indication that dopamine activity in his or her brain's reward system is especially sensitive to cocaine.^h
- An individual's attraction to cocaine's dopamine-stimulating effects also may relate to his or her social circumstances.ⁱ

a "Brain Scans Open Window to View Cocaine's Effects on the Brain," *NIDA Notes* 13-2.

b Venton, B.J., et al. Cocaine increases dopamine release by mobilization of a synapsin-dependent reserve pool. *The Journal of Neuroscience* 26(12):3206-3209, 2006. Article will be featured in *NIDA Notes* 21-5.

c "Animal Studies Suggest D3 Receptors Offer New Target for Treatment Medications," *NIDA Notes* 18-4.

d "Altered Cellular Activity May Be First Step in Progression to Cocaine Addiction," *NIDA Notes* 16-5.

e "Even Modest Cocaine Use May Cause Brain Changes That Could Contribute to Addiction," *NIDA Notes* 16-3.

f "In Chronic Drug Abuse, Acute Dopamine Surge May Erode Resolve to Abstain," *NIDA Notes* 19-1.

g "Stimulant Drugs Limit Rats' Brain Response to Experience," *NIDA Notes* 19-3.

h "Behavioral Response to Novelty Foreshadows Neurological Response to Cocaine," *NIDA Notes* 21-2.

i "Social Environment Appears Linked to Biological Changes in Dopamine System, May Influence Vulnerability to Cocaine Addiction," *NIDA Notes* 17-5.

SUMMARY

By altering neurotransmission, addictive drugs produce effects that make people want to continue to abuse them and induce health problems that can be long-lasting and profound. The effects are drug-specific: Each drug disrupts particular neurotransmitters in particular ways. Some important effects, however, are shared by all: initial pleasurable feelings, and subsequent dependence and addiction, resulting from disruption of the dopamine neurotransmitter system.

Scientists use a wide variety of experimental tools and techniques to study drugs' effects on neurotransmission, and their consequences, in both animals and people. Their findings enhance our understanding of the experiences of drug abusers and the plight of addicts, point the way to new behavioral and medication treatments, and provide potential bases for prevention strategies and monitoring progress in treatment. ■

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1 "Nicotine's Multiple Effects on the Brain's Reward System Drive Addiction," *NIDA Notes* 17-6.

2 "Genetic Engineering Studies May Lead to Development of More Effective Pain Relievers," *NIDA Notes* 15-3.

3 "Stimulant Drugs Limit Rats' Brain Response to Experience," *NIDA Notes* 19-3.

4 "Ecstasy Damages the Brain and Impairs Memory in Humans," *NIDA Notes* 14-4.

5 "Methamphetamine Abuse Linked to Impaired Cognitive and Motor Skills Despite Recovery of Dopamine Transporters," *NIDA Notes* 17-1.

6 "Nicotine Alters the Developing Rat Brain," *NIDA Notes* 21-2.

7 "Microscopic Probe Detects Changes in Brain Chemistry as They Occur," *NIDA Notes* 19-1.

8 "Brain Glutamate Concentrations Affect Cocaine Seeking," *NIDA Notes* 19-3.

9 "Nicotine Withdrawal Linked to Disrupted Glutamate Signaling," *NIDA Notes* 19-6.

10 "Smoking Decreases Key Enzyme Throughout Body," *NIDA Notes* 19-1.

Study Links Anabolic Steroids to Brain Changes in Adolescent Female Mice

Exposure may promote aggression in girls.

BY CARL SHERMAN,
NIDA Notes Contributing Writer

Anabolic androgenic steroid (AAS) abuse, once largely limited to elite athletes, has spread to a wider population that includes adolescents along with adults, and girls as well as boys. While the psychological and behavioral consequences of AAS use presumably reflect its impact on a number of brain areas, a NIDA-funded study at Dartmouth Medical School has identified one neurobiological effect that has potentially important implications for the emotional stability and well-being of adolescent girls in particular.

Principal investigator Dr. Leslie Henderson and colleagues studied the effect of the AAS, 17 α -methyltestosterone (17 α -MeT), on the activity of the neurotransmitter gamma-aminobutyric acid (GABA) in adolescent mice. Loosely speaking, GABA acts as a calming agent throughout the nervous system: It dampens activity of the neurons to which they are connected. Specifically, the researchers focused on the steroid's impact on GABA functioning in the medial preoptic area (MPOA) of the basal forebrain, a region that participates in the regulation of sexual behavior,

anxiety, and aggression. They found that in female, but not male, animals the AAS interfered with GABA transmission in the area. Theoretically, this effect would reduce GABA's inhibitory influence and thus potentially contribute to the excessive emotions and behaviors seen in AAS abuse. Various studies have linked increased anxiety and aggression, and both increased and decreased libido to AAS use.

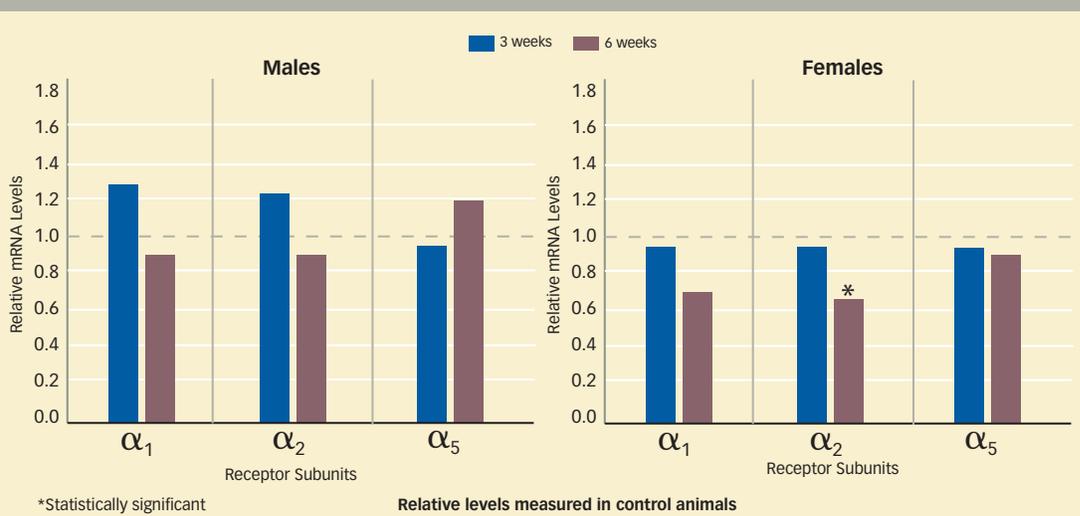
"The GABA system isn't the only target for the effects of AAS, but it is likely an important one," Dr. Henderson says. "Going into the experiment, we assumed we'd see an anabolic steroid effect on the GABA system in the MPOA and expected there would be differences between males and females." This area of the brain, particularly the cluster of neurons within it called the medial preoptic nucleus (MPN), is structurally different in the sexes.

DRUG TARGETS RECEPTORS

The researchers injected mice with a solution of 17 α -MeT in sesame oil, in doses (7.5 mg/kg/d) that would correspond to those taken by humans who are abusing the drug heavily. They injected a control group of mice with the sesame oil vehicle alone. The researchers examined brain tissue from half the mice in each group after 3 weeks of treatment and from the other half after 6 weeks. They focused on the subunits that make up GABA type A receptors (GABA_A) in cells of the MPN and on the way that AAS exposure affected the function of these receptors. Each receptor contains five of these subunits, proteins that determine the receptor's sensitivity to drugs and hormones.

To test how the reduction in α_2 subunit production might affect GABA_A receptor

EFFECTS ON RELATIVE LEVELS OF GABA RECEPTOR mRNA The researchers measured the effects induced by anabolic androgenic steroids (AAS) on GABA_A receptor subunit mRNA levels for male and female mice treated with 17 α -MeT for 3 (blue) or 6 (purple) weeks. Six weeks of AAS exposure decreased the amount of one of the subunits of the receptor α_2 only in female mice. No significant effects were observed in the treated male mice.



function, the researchers measured the amplitude and frequency of inhibitory postsynaptic currents (IPSCs)—a measure of the receptor’s efficacy in inhibiting the activity of neurons—in the MPN. Here, too, they found sex-based differences that were magnified by AAS. In untreated mice, the IPSCs were smaller in amplitude in females than in males. Female mice that received 3 to 4 weeks of AAS displayed smaller and less frequent currents than controls, suggesting that exposure to the drug had reduced GABA_A receptor function, thereby widening the gender gap. There was no comparable change in males.

The researchers concentrated on the $\alpha 2$ subunit family, which earlier studies had shown that 17 β -MeT alters. Before treatment, levels of messenger RNA (mRNA) for the $\alpha 2$ subunit were lower in female than in male mice in cells of the MPN. After 6 weeks, $\alpha 2$ subunit mRNA—an indicator of the quantity of the subunit being produced—had declined by 37 percent in female mice treated with the AAS compared with controls, but was essentially unchanged in males. When the researchers measured the actual protein that makes up the $\alpha 2$ subunit in female mice, they found a small but significant reduction (8 percent) in the number of neurons containing $\alpha 2$ protein.

Chronic exposure to of the AAS augmented gender differences in both the structure and function of certain GABA receptors, Dr. Henderson says. “Overall, the effect was to decrease GABA transmission in the MPN of female, but not male, adolescent mice. This would presumably increase the level of activity or change the pattern of activity in postsynaptic neurons of the female mice.”

A CLOSER LOOK

How do these neurobiological changes contribute to the behavioral manifestations of AAS abuse? “It could be that an AAS that promotes aggression in males would promote it more in females, or have different effects on the expression of sexual behaviors, but this is something we are just beginning to explore,” Dr. Henderson says. “What’s more, there are 60 to 100 AAS, and their neurobiological effects are unlikely to be uniform. In time, we may be able to start parsing out whether certain commonly abused steroids are likely to amplify aggression and libido in women or in men while others affect both genders equally.”

Dr. Henderson notes that alterations in GABA_A receptor function could have other important effects as well. “The GABA_A receptor is a major target of many drugs, including alcohol and benzodiazepines. Changing the subunit composition could

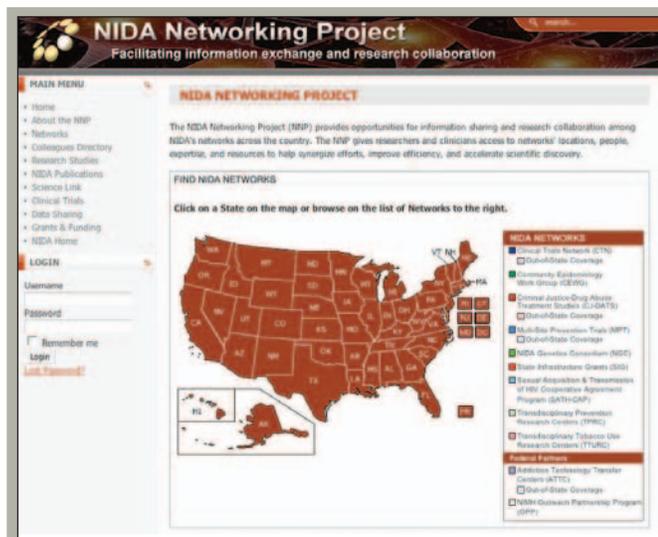
alter the brain’s sensitivity to these chemicals.”

“Although this is a basic research study, its potential translation to humans, even if speculative, is striking,” says Dr. Pushpa Thadani, formerly of NIDA’s Division of Basic Neuroscience and Behavioral Research. “It demonstrates that AAS exposure in the adolescent period produces gender-specific changes at the molecular level that may be correlated with known behavioral outcomes.”

Although applying findings from this and similar studies in actual interventions remains a distant goal, “these studies advance our understanding of the actions of AAS on the brain and behavior, which can empower us to better educate the lay public on the harmful effects associated with abuse,” Dr. Thadani says. “The research is still in its infancy,” she observes. Other studies, now under way, are seeking to clarify the links between aggression in female mice and AAS-associated neurobiological changes. “When these findings are available, we’ll probably be in a better position to translate this information into the human arena.”

SOURCE

Penatti, C.A.A., et al. Sex-specific effects of chronic anabolic androgenic steroid treatment on GABA_A receptor expression and function in adolescent mice. *Neuroscience* 135(2):533-543, 2005.



The NIDA Networking Project Web site provides access to information, expertise, and resources emerging from NIDA’s primary research and data collection networks. The information includes:

- Map locations and contacts for each network site
- Network descriptions and missions
- Links to network Web sites
- Links to scientific protocols and papers
- Links to procedural policies and manuals
- NIDA news and events of interest to networks
- NNP Colleagues Directory, a searchable database of members’ expertise and research focus

nnp.drugabuse.gov

Meeting Reviews Roles of Drug Abuse and Risky Behavior in HIV



More than 400 researchers and clinicians gathered in Bethesda, Maryland, May 8-9 to discuss the impact of drug abuse on the spread of HIV. NIDA sponsored the meeting in collaboration with other National Institutes of Health agencies and the Centers for Disease Control and Prevention.

Drug and alcohol abuse can prompt poor judgment and actions that people might not engage in otherwise, for example, risky sexual behaviors with infected partners. Drug abuse also may increase HIV transmission when abusers trade sex for drugs or money. The increased risk of HIV related to drug abuse extends beyond the sharing of drug injection equipment to include such risky behaviors.

Dr. Igor Grant of the University of California, San Diego, addressed methamphetamine abuse and HIV. The drug and the infection each cause degenerative brain disease. They may damage neural tissue through a common biological pathway—for example, enhanced inflammatory responses—but also seem to generate distinct pathologies that may combine to exacerbate each other. The resulting cognitive impairments may explain why methamphetamine abuse reduces adherence to HIV medications, that, in turn, may facilitate HIV transmission.

Dr. Jonathan M. Ellen of the Johns Hopkins University School of Medicine linked marijuana abuse with risky sexual behavior among African-American youth in San Francisco. Another study indicated that HIV-infected young women who abuse marijuana are less likely to keep medical appointments.

Dr. Richard A. Rawson of the University of California, Los Angeles, reported preliminary results suggesting that addiction treatment reduced risky sexual behaviors among methamphetamine abusers—adding to similar findings among methamphetamine-abusing men who have sex with men (see “Treatment Curbs Methamphetamine Abuse Among Gay and Bisexual Men,” *NIDA Notes*, Vol. 20, No. 4). Similarly, Dr. Kenzie L. Preston of NIDA’s Intramural Research Program reported that adding behavioral therapy to methadone maintenance treatment

can reduce risky behaviors among outpatients addicted to heroin and cocaine.

NIDA-funded research on the link between drug abuse and HIV infection extends to the international community. Dr. Steffanie A. Strathdee, an infectious disease epidemiologist at the University of California, San Diego, reported that HIV infection among injection drug users has risen sharply in many countries—including the Ukraine, the Russian Federation, Vietnam, Iran, and China—with emerging epidemics in other nations. Interventions that reduce risky behaviors among populations that interact with both high- and low-risk individuals (for example, prisoners, non-injecting drug users, and sex workers who inject drugs) are important opportunities for HIV prevention.

Participants also discussed the disproportionate burden of HIV/AIDS among people in prisons and jails and the importance of interventions in such settings. Improved technology now allows for faster HIV testing, and expanded testing and counseling are key components of prevention strategies for drug abusers and other groups.

Adolescent Inhalant Use Is Stable Overall, but Rising Among Girls

Almost 5 percent of girls between the ages of 12 and 17 used an inhalant to get high in 2005, an increase from 4.1 percent in 2002, according to a new report. Overall, inhalant use by boys and girls in this age group remained stable over the 4-year period, at an average annual rate of 4.5 percent, or an estimated 1.1 million adolescents.

The report, “Patterns and Trends in Inhalant Use by Adolescent Males and Females: 2002-2005,” is based on an analysis of the 2002-2005 National Household Survey on Drug Use and Health, an annual survey sponsored by the Substance Abuse and Mental Health Services Administration (SAMHSA). SAMHSA, NIDA, and other federal agencies released the report at a March 15 press conference to kick off the 15th National Inhalants and Poisons Awareness Week, March 18-25.

“Young people who turn to inhalants may be completely unaware of the serious health risks,” said NIDA Deputy Director Dr. Timothy P. Condon. “We know that inhalant abuse can start early, with research suggesting that even preadolescent children seek them out because they are easy to obtain. NIDA research also indicates that those who begin using inhalants at an early age are more likely to become dependent on them—and long-term inhalant abusers are among the most difficult drug abuse patients to treat.”

The report is available at www.drugabusestatistics.samhsa.gov/2k7/inhalants/inhalants.pdf.

Journal Highlights Global Nexus of Drug Abuse and HIV/AIDS

One of the most urgent public health goals of addiction researchers is to curb drug abuse behaviors that contribute to the spread of HIV/AIDS, says a special supplement to *Drug and Alcohol Dependence* sponsored by the NIDA International Program.

Dr. Steven Gust, director of the program and an editor of the supplement, observes that about 5 percent of the world's population aged 15 to 64 abuses drugs and that this behavior is a major factor in the transmission of HIV/AIDS and other illnesses. Two key routes of HIV infection are through the sharing of needles and other drug injection paraphernalia and high-risk sexual contact, with the latter being one of the fastest-growing routes among women.

NIDA-supported research has shown that preventive interventions and substance abuse treatment can reduce the transmission of HIV in drug-abusing populations. By promoting collaborative research across the globe, the NIDA International Program hopes to improve treatments and outreach programs to reduce high-risk drug use and sexual behaviors, Dr. Gust says.

The supplement compiles studies—several of them funded by NIDA—on drug-related HIV transmission in 16 different localities across the globe. Most of the studies focus on injection drug use, a primary pathway for HIV transmission. Some of the topics covered are:

- The relationships between needle-sharing practices and HIV infection among heroin abusers in Dar es Salaam, Tanzania;
- The prevalence of HIV and hepatitis C infections among injection drug users receiving substance abuse treatment in two large hospitals in Barcelona, Spain;
- HIV prevalence among heroin-addicted individuals in Muar, Malaysia;
- Sexual risk behaviors among injection drug users in Shanghai, China;
- The impact of drug abuse on adherence to highly active antiretroviral therapy (HAART) among HIV-positive outpatients in France;
- HIV/AIDS risk factors along the U.S.-Mexico border;
- Regional differences in the characteristics of injection drug users in New South Wales, Australia; and
- The acceptability of audio computer-assisted self-interview among substance abusers in Rio de Janeiro, Brazil.

Since 1990, the NIDA International Program has fostered cooperative research and the exchange of scientific information by drug abuse researchers worldwide. The program's objectives include promoting international research and collaboration, communicating and disseminating science-based drug abuse information, and supporting research training and exchange opportunities.

More information on the link between drug abuse and HIV/AIDS is available at www.hiv.drugabuse.gov.

SOURCE

Gust, S.W., Strathdee, S.A., and Winstanley, E.L., eds.. Drug Abuse and HIV/AIDS: International Research Lessons and Imperatives. *Drug and Alcohol Dependence* 82(S1), 2006.

NIDA at Your Fingertips

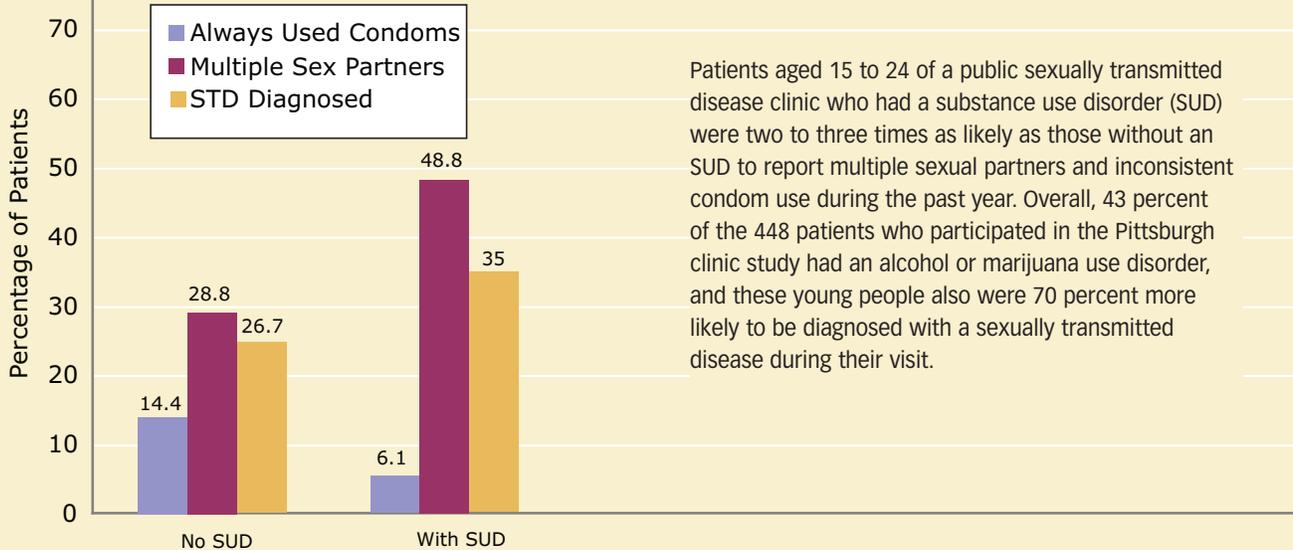
News and information about NIDA research, programs, and events is quickly and easily accessible through NIDA's home page:



www.drugabuse.gov

- Information on Drugs of Abuse
- Publications (including *NIDA Notes*)
- Calendar of Events
- Links to NIDA Organizational Units
- Funding Information
- Internal Activities
- Links to Related Web Sites

Among Young Attendees at STD Clinics, Substance Abusers Report More Risky Sexual Behavior



Patients aged 15 to 24 of a public sexually transmitted disease clinic who had a substance use disorder (SUD) were two to three times as likely as those without an SUD to report multiple sexual partners and inconsistent condom use during the past year. Overall, 43 percent of the 448 patients who participated in the Pittsburgh clinic study had an alcohol or marijuana use disorder, and these young people also were 70 percent more likely to be diagnosed with a sexually transmitted disease during their visit.

SOURCE: Cook R.L., et al. Alcohol and drug use and related disorders: An underrecognized health issue among adolescents and young adults attending sexually transmitted disease clinics. *Sexually Transmitted Diseases* 33(9):565-570, 2006.

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