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**

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."

NIDA Research Illuminates Associations Between Psychiatric Disorders and Smoking

By Patrick Zickler, NIDA NOTES Staff Writer

Nearly half of all cigarettes sold in the United States are sold to people with mental illness, and men and women with mental disorders are twice as likely as the general population to smoke. A recent NIDA-supported epidemiological analysis reveals relationships between psychiatric disorders and smoking that have important implications for public health. The findings suggest that treating psychiatric illness can contribute to reductions in smoking intensity and nicotine addiction, and that addressing smoking during substance abuse treatment is vital to counter an increased risk for nicotine addiction that may accompany recovery.

Dr. Naomi Breslau, at Michigan State University in East Lansing, used data from the Tobacco Supplement to the National Comorbidity Survey (NCS) to study the relationships between the temporal onset of psychiatric disorders, psychiatric symptoms, and smoking. The NCS, mandated by Congress to assess the prevalence of psychiatric disorders in the United States, surveyed a representative sample of the national population between 1990 and 1992, eliciting information about the onset of psychiatric disorders—as defined by the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition Revised (DSM-III-R)—and the time course of their symptoms. Disorders included in the NCS are major depression, dysthymia (similar to clinical depression, but with longer-lasting and milder symptoms), agoraphobia, generalized anxiety disorder, simple and social phobias, panic disorder, posttraumatic stress disorder, and alcohol or drug abuse or addiction. The NCS Tobacco Supplement asked respondents whether they smoked, when they began smoking daily, at what age they experienced symptoms matching DSM criteria for nicotine dependence, and whether they had stopped smoking regularly a year or more before they took part in the survey.

<p>| Active Psychiatric Disorders Increase Likelihood of Daily Smoking, Nicotine Addiction |
|-------------------------------------------------|-----------------|-----------------|
| Relative Risk of Transition to Daily Smoking | Relative Risk of Developing Nicotine Addiction |</p>
<table>
<thead>
<tr>
<th>When Symptomatic</th>
<th>When Remitted</th>
<th>When Symptomatic</th>
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<tbody>
<tr>
<td><strong>Depressive Disorders</strong></td>
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<tr>
<td>Major Depression</td>
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<td>2.2</td>
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<tr>
<td>Dysthymia</td>
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<td>1.5</td>
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<td><strong>Anxiety Disorders</strong></td>
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<td>1.8</td>
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<td>GAD</td>
<td>2.1</td>
<td>NE</td>
<td>1.8</td>
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<tr>
<td>Simple Phobia</td>
<td>1.5</td>
<td>0.9</td>
<td>1.8</td>
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<td>2.8</td>
<td>1.8</td>
</tr>
<tr>
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<td>1.7</td>
<td>1.4</td>
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<tr>
<td>Alcohol A/D</td>
<td>1.5</td>
<td>0.5</td>
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Researchers found that active psychiatric disorders, with the exception of agoraphobia and panic disorder, were associated with increased risk of transition to daily smoking. In contrast, past disorders (those that had been inactive for a year or more) generally did not predict transition to daily smoking. Researchers also found an increased risk of transition to nicotine addiction associated with a wide range of active disorders, but only four past disorders. Risks are presented as odds ratios; a relative risk of 2.0 indicates twice the likelihood.

Analyzing the responses from 4,414 survey participants, Dr. Breslau found that:

- Men and women with histories of substance abuse, major depression, and most anxiety disorders reported increased rates of transition to daily smoking, but only during periods when they were experiencing symptoms. When their illnesses had been asymptomatic for a year or more, they became daily smokers at rates no higher than respondents who never experienced psychiatric illness;
- Substance abuse and major depression predicted transitions from voluntary smoking to nicotine addiction when actively symptomatic (the association was borderline for drug, as opposed to alcohol, abuse). In the case of substance abuse, this relationship became markedly stronger when the problems had remitted for at least a year;
- Most anxiety disorders increased risk for nicotine addiction when symptomatic. For individuals with simple phobia or panic disorder, these risks multiplied during periods of remission. For those with posttraumatic stress disorder, the risk reverted to baseline when symptoms had been absent for a year; and
- None of the psychiatric disorders studied affected respondents' chances of successfully quitting smoking, either when active or when remitted.

"We found that the majority of the psychiatric disorders, when active, predicted the onset of daily smoking," Dr. Breslau says. "Respondents with one active disorder were 1.3 times as likely, and those with four or more active disorders were 2.2 times as likely to begin daily smoking as those with no active disorders. This suggests that early treatment may be able to prevent patients who are not currently daily smokers from progressing to that status."

"Similarly," Dr. Breslau says, "most disorders—when active—predicted that smokers would progress from daily smoking to nicotine addiction. In this transition from one stage of smoking to another, daily smokers with one active disorder were on average 1.8 times as likely as those with no active disorder to develop addiction, and the odds of developing nicotine addiction increased with the number of active disorders. This suggests that successful control of psychiatric symptoms before smokers become addicted can prevent them from making that transition."

Substance abuse, however, is an important exception to this general observation. Respondents with past but not active alcohol and drug abuse disorders had risk ratios two to three times as high as respondents with current active disorders involving these substances. "This association suggests that cessation of substance abuse may induce greater smoking intensity. In treatment for substance use disorders it is important to be conscious of smoking behavior, to guard against the possibility that a person in treatment for one damaging condition might increase the danger posed by another. Treatment should assist patients who are abusing alcohol or drugs and who smoke to quit both," Dr. Breslau says.

When Dr. Breslau looked for a relationship between mental disorders and quitting smoking, she found that neither active nor remitted disorders made respondents more or less likely to quit smoking successfully during the year preceding the survey.

"The relationship between tobacco use and comorbid psychiatric disorders is complex," says Dr. Kevin Conway of NIDA's Division of Epidemiology, Services and Prevention Research. "While we see variations across the range of disorders included in the comorbidity survey, the consistent pattern in this study emphasizes the importance of active expression of psychiatric disorders—not simply a history of the disorder—in relation to smoking stages."

<table>
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<tr>
<th>Drug A/D</th>
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<th>0.9</th>
<th>1.5</th>
<th>4.1</th>
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<td>GAD indicates general anxiety disorder; NE, not evaluated; PTSD, posttraumatic stress disorder; and A/D, addiction/dependence.</td>
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Source

Nicotine Withdrawal Linked to Disrupted Glutamate Signaling

By Patrick Zickler, NIDA NOTES Staff Writer

More than a third of America's 46 million adult smokers try to stop each year, but fewer than 10 percent succeed. Some relapse because they cannot tolerate the discomfort and craving associated with nicotine withdrawal. In recent animal studies, NIDA-supported scientists identified sites on some brain cells that appear to be key promoters of the negative psychological symptoms of nicotine withdrawal. The sites, called glutamate receptors, are part of the communication network that uses the neurotransmitter glutamate as a chemical messenger.

Neurobiologists have previously shown that glutamate helps produce the good feelings smoking causes. When nicotine attaches to receptors on cells in the brain's ventral tegmental area (VTA), the cells release glutamate, which in turn triggers other VTA cells to release dopamine, a neurotransmitter that produces pleasure. Dr. Athina Markou of The Scripps Research Institute (TSRI) in La Jolla, California, and colleagues reasoned that just as glutamate surges caused by nicotine give rise to smoking pleasure, glutamate depletion related to nicotine abstinence might underlie the displeasure of withdrawal. The researchers speculated that when nicotine is withdrawn after chronic use, the feedback system that restores glutamate to normal levels following surges could overshoot its mark, resulting in a glutamate dearth—and symptoms of depression and irritability.

To test this idea, Dr. Markou and Dr. Paul Kenny at TSRI, along with Dr. Fabrizio Gasparini of Novartis Institutes for Biomedical Research in Basel, Switzerland, focused on a specific group of glutamate receptors called group II metabotropic glutamate (mGluII) receptors. These inhibitory receptors are key components of the glutamate

Asking a Rat, "How Do You Feel?"

Drug-Dependent Rat

Receives pleasurable stimulation via an electrode

Learns that wheel-turning earns another stimulation

The lowest level of stimulation required to motivate the rat to turn the wheel is baseline "reward threshold"

When the rat becomes uncomfortable for any reason, the reward threshold rises proportionately (it takes more stimulation to provoke wheel-turning)

If the discomfort subsides, the reward threshold returns rapidly toward the baseline

Dr. Athina Markou and her colleagues used this experimental technique, known
feedback system: They detect high glutamate levels and signal glutamate-producing cells to reduce their activity to bring the levels back down. Inactivating the mGluII receptors interrupts this process, leaving glutamate levels high. The researchers hypothesized that if they inactivated rats' mGluII receptors while subjecting the animals to nicotine withdrawal, the plunge in glutamate levels may be avoided, and the animals' withdrawal symptoms attenuated.

The scientists implanted tiny pumps under the skin on the backs of adult male rats. The pumps dispensed a nicotine solution that maintained high nicotine levels equivalent to those produced in a human who smokes 30 cigarettes per day. After the rats had been exposed to nicotine for 7 days, the investigators removed the pumps, depriving the animals of nicotine and thus leading to nicotine withdrawal. Then, after 18 hours of withdrawal, half the rats were injected with a chemical that blocks the action of mGluII receptors, in effect switching off the inhibitory feedback signals to the glutamate-producing cells. Over the next 72 hours the scientists evaluated the rats at regular intervals using a technique, called intracranial self-stimulation (see "Asking a Rat, 'How Do You Feel?'"), that measures withdrawal-like depression in laboratory animals. As the scientists had predicted, the rats with active mGluII receptors exhibited significant discomfort; the withdrawal discomfort rapidly dissipated in those in which mGluII receptors were turned off.

To help confirm the association between mGluII receptors and withdrawal-like symptoms, Dr. Markou's team treated nicotine-dependent rats with a compound that stimulates the same receptors. In these animals, activation of the inhibitory glutamate loop triggered discomfort comparable with that in nicotine withdrawal.

"Other research has shown how nicotine changes regulation of excitatory glutamate signaling," Dr. Markou says. "Our study helps explain how nicotine also commandeers inhibitory glutamate circuits. The altered function of the mGluII receptors appears to mediate, at least partly, the depression-like aspects of nicotine withdrawal." The effect, she explains, is a carrot-and-stick influence strong enough to thwart the most sincere attempts to quit smoking. "Nicotine provides a positive effect through the excitatory circuits, making smoking a rewarding and reinforcing experience. Now we see that nicotine has a similarly powerful aversive effect through the inhibitory circuits, making withdrawal an unpleasant experience."

The role of mGluII receptors in withdrawal suggests that these receptors might also offer a target for therapeutic intervention, Dr. Markou adds. "Easing the depression-like aspects of withdrawal would significantly decrease discomfort and make it easier for people to maintain abstinence and resist the temptation to relapse to smoking."

Source:

- Kenny P.J.; Gasparini, F.; and Markou, A. Group II metabotropic and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA)/kainate glutamate receptors regulate the deficit in brain reward function associated with nicotine withdrawal.

[Full Text]
Heavier Maternal Smoking During Pregnancy Increased Children’s Odds of Nicotine Addiction as Adults

Children of women who smoked at least 20 cigarettes a day during pregnancy were more likely to become addicted to
nicotine or progress from regular smoking to nicotine addiction as adults compared with children of women who smoked fewer than 20 cigarettes a day. Children of heavier smokers were no more likely to try smoking or to smoke regularly than children of lighter smokers.

Maternal Tobacco Smoking During Pregnancy Did Not Affect Children’s Odds of Marijuana Use as Adults

The finding that in utero exposure to tobacco did not affect later marijuana use indicates that the two drugs have different physiological pathways.

Volume 19, Number 4 (December 2004)

The evidence from this study, which reinforces the findings of experimental research with animals, is compelling,” says Dr. Buka. “Early exposure to tobacco during pregnancy apparently affects the individual’s response to cigarettes in later adolescence and adulthood.”

The researchers' statistical analyses indicated that the associations between maternal smoking during pregnancy and offspring's future smoking were independent of socioeconomic status, maternal age at pregnancy, offspring sex, and offspring age at the time of the interview. What's left, then, is a biological factor. "The most likely hypothesis is that the toxins in cigarettes cross the placental barrier and interact with the genes that control cell differentiation, permanently altering cells' responsiveness in ways that increase vulnerability to tobacco addiction," Dr. Buka says.

The cross-generational impetus to tobacco addiction documented by the study is a serious national health concern. Almost half of women who smoke continue to do so when they become pregnant, says Dr. Buka. The smoking mothers-to-be constitute about 12 percent of women who give birth—a national potential for 500,000 prenatal exposures every year.

The researchers also collected information about the study participants' marijuana abuse and found no tie to prenatal nicotine exposure. This suggests, the investigators say, that the "pathophysiological pathway" that promotes vulnerability to tobacco addiction among offspring differs from the pathway that leads to marijuana addiction.

The study confirms the need for energetic efforts to deter women from smoking, especially during pregnancy, says Dr. Kevin Conway, deputy chief of NIDA’s Epidemiology Research Branch. Preventing smoking by pregnant women will improve nicotine addiction rates of the next generation. "This study highlights opportunities for physicians to intervene with mothers who smoke, for the health of themselves and their children," says Dr. Conway.

"Healthy-baby prenatal visits, labor and delivery, and postnatal-care visits are golden opportunities for providers to offer assistance to quit smoking and prevent relapse, thereby reducing the risk of children's progression to nicotine addiction," says study coauthor Dr. Niaura. "Health care providers must take advantage of every opportunity to ask, advise, and assist patients in efforts to quit smoking."

Source


Abstract