Research Findings
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Vaccine May Reduce Fetal Exposure to Nicotine

Antibodies that block nicotine's path across the blood brain barrier may also inhibit placental absorption.

BY CARL SHERMAN, *NIDA Notes* Contributing Writer

Vaccine-induced antibodies that facilitate smoking cessation by blocking nicotine penetration into the brain also markedly reduce the drug's passage across the *ex vivo* human placenta, a NIDA-funded study has demonstrated. The finding suggests that maternal immunization during pregnancy may be safe and may to some extent protect the fetus from exposure to nicotine.

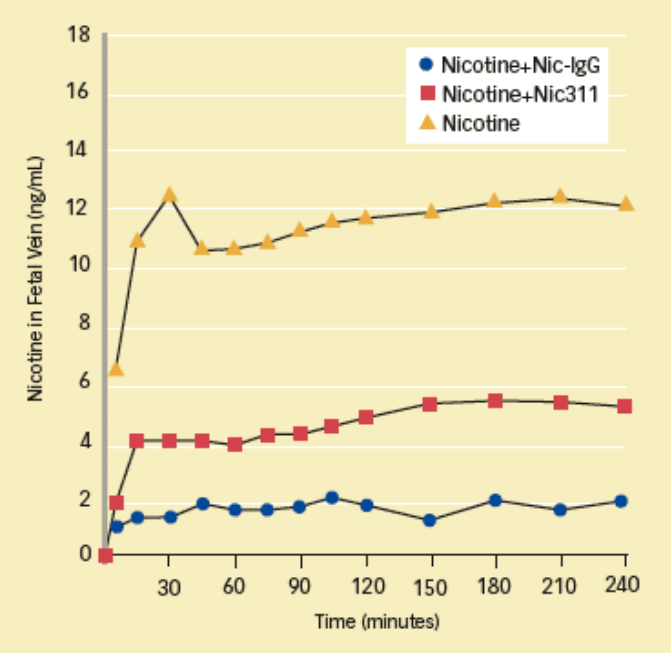
The adverse effects of maternal smoking during pregnancy include increased rates of miscarriage, premature delivery, low birth weight, neonatal mortality, and sudden infant death syndrome (SIDS). Increasingly, research has linked prenatal smoking exposure to children's neurobehavioral problems, such as attention deficit-hyperactivity disorder. The role of nicotine in causing this damage is not entirely clear, but animal studies suggest the drug may compromise fetal development directly or through its effects on the placenta. "We desperately need medications that can help women quit smoking during pregnancy, medications that are both effective and do not themselves harm the fetus. This study supports the potential use of immunization," says Dr. Paul Pentel of the University of Minnesota Medical School, one of the investigators.

THE EXPERIMENTAL PROCEDURE

NicVAX, a vaccine being developed by Florida-based Nabi Biopharmaceuticals with NIDA support, joins the nicotine molecule to a protein. The resulting molecule provokes the production of antibodies that combine with circulating nicotine to create a complex molecule that is too large to cross the blood-brain barrier. When the amount of nicotine reaching the brain drops far enough, the concept goes, "the smoker will no longer get a rewarding effect and will quit," says Dr. Scott Winston, a Nabi researcher. A recent small-scale clinical study found doserelated improvements in 30-day quit rates among 68 immunized smokers ("Nicotine and Cocaine Vaccines Move Forward").

The two antibodies used in the placenta transfer study, nicotine immune globulin (Nic-IgG) and a monoclonal antibody (Nic311) were taken, respectively, from rabbits and mice that produced them in response to

RESULTS OF MEASURING THE CONCENTRATION OF NICOTINE IN THE FETAL VEIN The addition of a nicotine-specific antibody significantly reduces the appearance of nicotine in the fetal vein. Of two antibodies tested, Nic-IgG was more effective than NIC311.



immunization with NicVAX. The research team, headed by Dr. Mahmoud Ahmed of the University of Texas Medical Branch, Galveston, tested the antibodies' effects on placental tissue and cross-placental nicotine transfer using a method developed in the mid-1980s: An intact lobule was dissected from placentas taken immediately after delivery and placed in phosphate-buffered saline. The researchers inserted catheters into blood vessels on the maternal and fetal side of the placental lobule and perfused each with tissue culture medium from a separate reservoir, creating distinct maternal and fetal circuits. They monitored placental function and viability for 2 hours, and then added nicotine to the fluid in the maternal reservoir. "We used a concentration (40 ng/mL) that has been reported in the circulation of mothers who smoke," Dr. Ahmed says. Either Nic311 or Nic-IgG along with nicotine was added to the maternal reservoir. Following these infusions, the researchers continued to monitor placental tissue health and tracked nicotine and antibody concentrations in both maternal and fetal circuits for 4 more hours.

SAFETY REASSURANCE

"Our primary interest in these studies was vaccine safety: Would it be safe to vaccinate women who may become pregnant, or during pregnancy? Antibodies might protect the fetus, but we also worried that they might escort nicotine across the placenta or sequester it in the fetus, increasing exposure," says Dr. Pentel. "The studies look reassuring."

When nicotine alone was added to the maternal circuit, it readily crossed the placenta; its concentration in the fetal circuit increased rapidly over the first 30 minutes. It did not change in the next 210 minutes. The addition of either antibody markedly reduced the rate at which nicotine crossed the placenta. With Nic311, nicotine reached a concentration of 1.8 ± 0.8 ng/mL in the fetal circuit in the first 5 minutes—about one-fourth of the transfer in the absence of the antibody. There was no significant increase in fetal circuit nicotine after the first 30 minutes. Nic-IgG had an even more pronounced effect: The concentration of nicotine in the fetal circuit was about one-half what it had been with Nic311 after the first 5 minutes (1.0 ± 0.04 ng/mL); it, too, rose little after that. Both antibodies also reduced the amount of nicotine retained in placental tissue.

"There was no effect of nicotine or either antibody on placental function or viability," Dr. Ahmed says. No appreciable amount (less than 1 percent) of either antibody appeared in the fetal circuits at any point in the experiment, suggesting that placental transfer was negligible.

Whether vaccination would protect the fetus from nicotine if a mother continued smoking is not yet clear. "I'm not sure that the effect would be large enough," Dr. Pentel says. Previous animal studies in which he was involved found that while antibodies sharply slow the rate at which a single dose of nicotine reaches

the brain, they do not stop the process altogether. "When nicotine is administered chronically in a way that approximates daily smoking, its long-term accumulation in the fetal brain looks the same in vaccinated and unvaccinated animals." Vaccination of pregnant rats reduced nicotine transfer to the fetal circulation and brain for 25 minutes after a single dose, but did not change accumulation in the fetal brain when nicotine was administered chronically. In another study, maternal vaccination did not prevent nicotine-induced upregulation of nicotinic cholinergic receptors or changes in gene expression (c-fos) in the fetal rat brain, Dr. Pentel observes. The *ex vivo* system used in the current study is not intended to model the effects of continual daily smoking, he says, but rather provides insight into the shorter term effects of antibodies on nicotine transfer across the placenta, as well as placental viability.

Dr. Amrat Patel, of NIDA's Chemistry and Pharmaceutics Branch, says the current study represents an important advance beyond animal research in suggesting that nicotine-specific antibodies can reduce placental transfer of nicotine in humans as well, but more work is needed to know whether the effect will be sufficient to prevent neurotoxicity. "We need to determine how much nicotine is necessary to cause fetal damage, and how to make sure nicotine does not approach that level." Antibodies with higher affinity for nicotine may make a difference, he says; as vaccine research continues, "we'll probably progress to develop antibodies that are even better able to sponge up nicotine."

SOURCE

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Director's Perspective
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Genes and Smoking

By NIDA Director, NORA D. VOLKOW, M.D.

Most of the 44.5 million American adults who smoke cigarettes would prefer not to. Why do so many would-be quitters fail, even with the help of stop-smoking interventions like nicotine replacement? Why, for that matter, do people become addicted to smoking in the first place? The answers lie partly in our genes.

NIDA researchers in collaboration with Perlegen Sciences, Inc., a private company, recently completed a search of the entire human genome for differences between individuals who are nicotine-dependent and those who smoked but never became dependent. Their target: single nucleotide polymorphisms (SNPs), locations on the genome where individuals differ by just one chemical unit in the makeup of their DNA. From 2.2 million known SNPs, researchers have identified roughly 40 to 80 that are highly correlated with nicotine addiction.

Once researchers link an SNP statistically to drug abuse, the question becomes: Does the gene do anything that might explain why people with one of its forms are more vulnerable to drugs than people with another? Some of the genes researchers have implicated in addiction affect the dopamine reward circuit. Others involve neurotransmitter systems and neural pathways not previously known to figure in smoking's effects. Researchers will use techniques such as brain imaging to correlate genetic differences with differences in brain structure or function and psychological tests to match them to behavior. Findings from the genome exploration may ultimately yield novel, more effective interventions.

Genetic variations can only partly explain why people become addicted to nicotine: A person's genetic makeup, experiences, and surroundings all combine to determine whether he or she will smoke and, if so, how difficult quitting will be. NIDA-funded epidemiologists and behaviorial scientists are conducting a large longitudinal study to elucidate these interactions. They have been following pairs of twins, now 17 years old, collecting information about participants' smoking and environmental factors like stressors and peer relationships that can increase the risk of substance abuse or protect against it. The next step will be to analyze these data, together with information on the twins' genetic and biological traits.

NIDA-supported researchers are also working to discover why interventions like

nicotine replacement therapy (NRT) work for some people and not others. By comparing smokers who have successfully used pharmacotherapy with those whose efforts to quit have failed, the researchers hope to identify groups of genes that predict who will do well with NRT, with bupropion, or with varenicline, the newest smoking cessation drug. The ultimate goal is to tailor the treatment to the smoker. Ultimately, we hope genetic studies will lead to strategies that protect vulnerable young people from addiction.

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Research Findings
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Nicotine Alters the Developing Rat Brain

Exposure to the drug during gestation or adolescence may cause lasting alterations in reward and motivation circuits.

BY CARL SHERMAN, *NIDA Notes* Contributing Writer

Most people who become chronic smokers start in adolescence, and the risk of addiction at this time is even greater among those whose mothers smoked while pregnant. NIDA-funded animal studies recently identified two neurobiological effects of nicotine that could underlie these vulnerabilities. Investigators at the University of Tennessee, led by Dr. Burt Sharp, found that prenatal nicotine exposure reduces the availability during adolescence of a receptor that mediates the drug's impact on cells in the brain's reward system. At the University of Wisconsin, Dr. Charles Landry and his research team found that nicotine stimulates a set of genes involved in synapse formation to a higher level of activity in adolescent than in adult rats.

NICOTINE'S IMPACT ON RECEPTORS

The University of Tennessee researchers pursued a clue from previous work in which they examined the effects of prenatal nicotine exposure on the mesolimbic reward pathway. Nicotine and other drugs of abuse stimulate neurons in the brain area where this pathway originates, the ventral tegmental area (VTA), to release the neurotransmitter dopamine in the nucleus accumbens (NAc) and prefrontal cortex (PFC). The dopamine influx into the NAc produces the feelings of reward and pleasure that are primary motivators of continued drug-taking. Dr. Sharp and colleagues found, however, that exposing rats prenatally to nicotine reduced the amount of dopamine released in the NAc when the animals were given the drug again as adolescents.

LOWER BINDING CAPACITY SUGGESTS LOWER NICOTINE REWARD The capacity to bind epibatidine is a marker for the concentration of nicotinic cholinergic receptors in a tissue sample. In the brain regions tested, this capacity was significantly lower in adolescent rats that had been exposed to nicotine during gestation. This suggests that prenatal exposure reduces later nicotine sensitivity in a brain circuit believed central to the drug's rewarding effect.

	Male		Female	
	Control	**Nicotine**	**Control**	**Nicotine**
Binding capacity (fmol/mg protein)				
NAc	38.2	30.2	37.7	27.1
PFC	55.1	45.6	55.7	41.9
VTA	59.5	43.3	45.9	39.3

"We asked ourselves, 'What causes this?'" Dr. Sharp says. "We decided to look at nicotine's impact on the expression of nicotinic cholinergic receptors—the principal sites where nicotine molecules interact with brain cells to exert their stimulating effects." The rese*arc*hers hypothesized that exposure to nicotine during gestation would reduce the number of such receptors present on dopamine-producing cells in the VTA in adolescence.

They gave nicotine to pregnant rats via an implanted pump at the rate of 2 mg/kg/day (the equivalent of a human smoking a pack a day) throughout gestation. At birth they increased the nicotine infusions to 6 mg/kg/day and continued them for 2 more weeks, while the rat pups nursed. Because rat pups are born at an earlier stage of development than humans, the weeks of continued exposure were necessary to give them cumulative nicotine exposure equivalent to a smoking mother's baby at full-term. A control group of rats received the nicotine delivery solution without the drug.

The rese*arc*hers took brain sections from the rat pups when they were 35 days old, developmentally equivalent to mid-adolescence in humans, and assayed them for nicotinic cholinergic receptors. In confirmation of their hypothesis, the results showed significantly fewer receptors in the VTA, NAc, and PFC of the adolescent rats that had been exposed to nicotine *in utero*. Messenger RNA (mRNA) for the receptors declined only in the VTA, suggesting that gestational nicotine had primarily affected dopaminergic neurons that originate in that area. The total number of VTA neurons also dropped in the brains of nicotine-exposed rats.

These findings "show how gestational exposure to nicotine may alter maturation, literally changing the brain," Dr. Sharp says. Although it is not clear how such changes could enhance the likelihood of dependence, "one hypothesis might be that prenatally exposed adolescents, having fewer nicotinic receptors, must take more puffs to release a rewarding amount of dopamine into the NAc, and this leads to stronger conditioning," he says. Dr. Allison Chausmer of NIDA's Division of Basic Neuroscience and Behavioral Rese*arc*h says, "The findings confirm the long-term effect of smoking during pregnancy and underscore the importance of smoking cessation at this time."

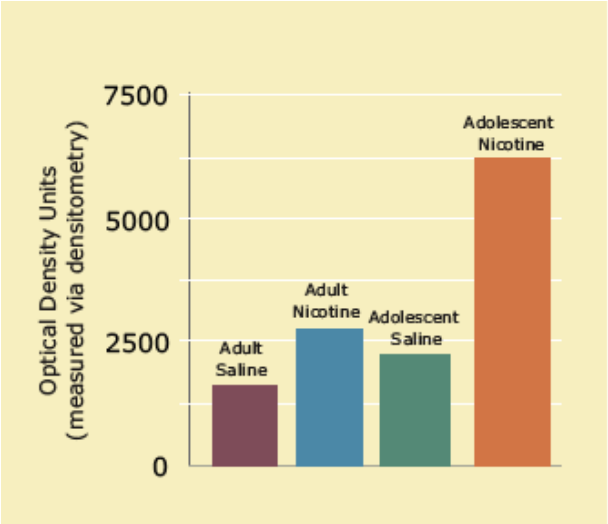
NICOTINE AFFECTS SYNAPSE DEVELOPMENT

The University of Wisconsin team studied the impact of nicotine on genes that contribute to neural plasticity. This process—the formation of new synaptic connections between neurons and pruning of old ones—wires the brain during development and reaches a crescendo during adolescence. The rese*arc*hers specifically focused on the genes—including *arc*, *c-fos*, and *NGFI-B*—that produce a set of neurochemicals involved in building synapses. Using rats as subjects, they compared the expression—roughly, the production rate—of these genes following exposure to nicotine in adolescents (average age 30 days) and adults (average age 70 days).

The investigators injected the rats with nicotine at a dose large enough (0.4 mg/kg) to cause a behavioral response —increased motor activity—or with saline. An hour later, they examined slices of the rats' brains, with particular attention to areas that play central roles in learning and motivation: the medial PFC, ventral and lateral

***arc* EXPRESSION INCREASES WITH NICOTINE** The height of the bars represents the expression of *arc*, a gene involved in neural plasticity. Administration of nicotine increases *arc* in the ventral and lateral cortex of both adolescent and adult rats, but significantly more in adolescents. This suggests that nicotine triggers synaptic development—a key process in learning—in a region important in motivation and goal-directed activity. Because the effect is greater in adolescents, they may more readily "learn" the nicotine habit.

orbital cortex (VLO), cingulate cortex, somatosensory cortex, ventral striatum, and dorsal striatum. They assessed the expression of plasticity-related genes by measuring the amount of their corresponding mRNA.



Throughout the brain, they found higher amounts of mRNA for *arc* and *c-fos* in the adolescent than the adult brains, an indication of more synaptic plasticity overall, Dr. Terri Schochet suggests. In both age groups, *arc* and *c-fos* mRNA jumped after injection of nicotine, compared with saline, indicating that the drug "switched on" these genes. In certain prefrontal regions, the nicotine-evoked increase in *arc* mRNA was significantly greater in adolescent animals. In the VLO, for example, *arc* expression increased by 182 percent in adolescents after nicotine injection, compared with 98 percent in adults.

"These findings show that at the basic biochemical level, the adolescent brain responds differently to a single dose of nicotine," says Dr. Landry, principal investigator for this study. "The enhanced expression of *arc*, a gene involved in dendrite formation, in adolescent forebrains following acute nicotine reflects a very dynamic synaptic milieu. It's difficult to speculate further, but my suspicion is that the adolescent brain responds to the drug with a greater increase in synaptogenesis and pruning."

"The adolescents' greater changes in molecular systems involved in learning may indicate that this age group is more susceptible to developing the nicotine habit," Dr. Schochet suggests. The striking effect of a single dose of nicotine could have implications for treatment, she adds: "It's really important to intervene as early as possible to prevent adolescents from trying nicotine in the first place."

Rese*arc*h that explores and compares adult and adolescent behavior and neurobiology is a particular interest of NIDA's, says Dr. Susan Volman of the Institute's Division of Basic Neuroscience and Behavioral Rese*arc*h. This study was valuable because it "looks at both what's different in general between the maturing and adult brain and how that difference interacts with nicotine."

Dr. Volman notes that the adult/adolescent disparity in response to nicotine was greatest in the ventrolateral PFC. "Neural adaptations here could have to do with altering motivation and the value placed on particular rewards," she says. Smoking might be equally pleasurable to adults and adolescents, that is, but the experience would be more highly valued by the adolescent—a difference with potential implications for tailoring behavioral treatments to this age group.

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