SEEK about TOBACCO

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Science Education Enhances Knowledge about TOBACCO

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A Brief Description

Science Education Enhances Knowledge (SEEK) about Tobacco is a unit containing 3 modules that provides science-based health and biology education regarding nicotine and tobacco products. The unit is a resource for educators and students to learn more about the science behind nicotine addiction, and the contributions by various kinds of tobacco products to the development of cancer.

SEEK about Tobacco is designed to be used in 2-3 class periods in a high school health education class or biology class. The unit uses a problem-based learning approach provided by the student activities at the beginning of each module. Answers to the "problem" are found in the "Content" section of each module.

Throughout the unit, there are bolded words, which are defined in the glossary. In addition, online resources are found under the "RESOURCES" list.

Last, Module 2 contains a lab experiment ("Can Tobacco Cause Mutations in DNA?"), which is conducted "virtually" after viewing a 10 minute video online (see the "Virtual Lab Video" at www.rise.duke.edu/seek). The lab is an important component of the unit, and the students should find it to be fun and informative.

SEEK about Tobacco was developed by scientists and faculty at Duke University Medical Center and the Duke University Fuqua School of Business with a SEDAPA grant (R25-DA23144) from the National Institute on Drug Abuse.

SEEK about Tobacco is a resource offered by Duke University's Raising Interest in Science Education (RISE) office. It can be viewed free of charge or downloaded as a PDF at www.rise.duke.edu/seek.

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The Addictive Nature of Nicotine

Content in this module will help students gain a scientific understanding of the addictive nature of nicotine in tobacco products. Addiction is a disease of the brain, and in the case of nicotine, it is very difficult to overcome.

The specific concepts covered in this module include:

- The toxicity of nicotine
- Modes of nicotine entry into the body
- Nicotine effects on brain function
- Activation of the brain's reward pathway
- The addiction process
- Differences between a teenager and an adult brain

Learning Objectives:

After completing this module, it is expected that students will be able to:

- List the modes of entry into the body for nicotine
- Trace the path that nicotine travels throughout the body
- Explain how neurons communicate with each other
- Name the neurotransmitter system activated by nicotine
- Describe the effects of nicotine on body functions
- Identify the major change that take place in the tobacco user's brain over time
- List and describe the steps leading to nicotine addiction
- Discuss the role of the reward pathway in the brain
- Explain the differences between an adult and teen's brain

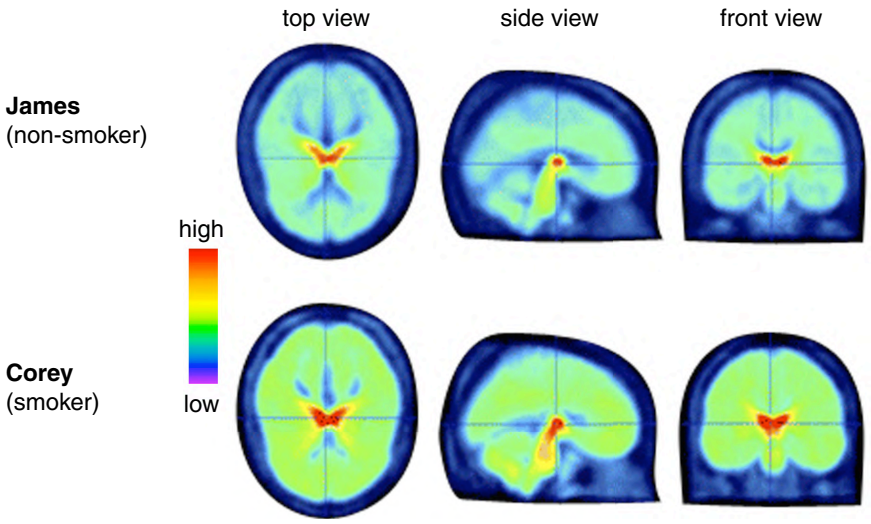
Story #1

Can Nicotine Really Change My Brain?

Corey and James are Juniors in high school…..Corey is a smoker, but James doesn't smoke. Their school nurse just announced that the local medical center is conducting a study on adolescent smokers to determine if nicotine, the main active ingredient in tobacco, can change the brain. Brain changes occur in the adult, but similar studies in adolescents are still lacking. The nurse mentioned that volunteers (both smokers and non-smokers) would receive $200 for participating in the study, so Corey and James decided to sign up.

When they got to the clinic, the research doctor in charge of the study told them that the study required getting a brain scan, called a PET scan (it wouldn't hurt, and it's not a dog scan!). The doctor told the boys that the PET scan of the brain shows the amount of specific proteins in brain cells to which drugs can attach. In this case, these proteins are called nicotine receptors, because nicotine binds to them, affecting the function of cells on which they reside. The scientists will look at the scans to compare the amount of nicotine receptors in Corey and James's brain.

So, here's what happened. Take a look at Corey and James's brain scans.



The blue areas show the lowest amount of nicotine receptors and the red areas show the highest amount of nicotine receptors. Can you tell the difference between the 2 boys? Corey, the smoker, has a lot more nicotine receptors in certain parts of his brain than does James. The same thing happens in adults--it's not a coincidence. So, how does something like this happen? And what are the consequences? To answer these questions, it will require some detective work on your part.

First, you'll need some background about the boys. Corey started smoking when he was 12 because his older brother Jeff (age 15) would sneak cigarettes behind the house. Corey and his friend James decided to try Jeff's stash one day when he wasn't around. The first puff was horrible – both Corey and James gagged and coughed. Corey finished smoking the cigarette because he thought it was "cool", but James didn't like it and didn't smoke any more.

Corey continued sneaking cigarettes from his brother when he could, but eventually he started wanting to smoke more often. When he didn't have a cigarette, Corey was a little anxious, but that went away after he smoked. It wasn't long before Corey realized he was "hooked"…he thought he could stop smoking any time, but he was never able to actually do it. So, you might not be surprised that Corey was interested in getting the PET scan to see if something was happening in his brain.

Your first "mission" is to become an expert about nicotine and the process of addiction. This becomes important later on as you'll see in the next module. Consult the Module 1 guide to answer the questions below for Mission #1.

Name _____ Group #_____

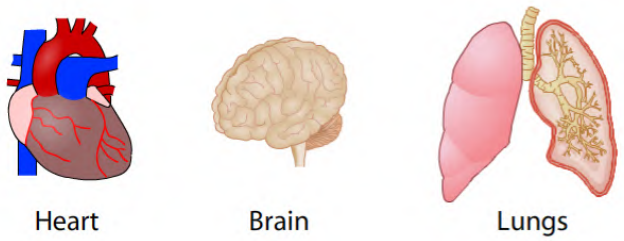
Mission #1

Answer the following questions:

1. Suppose Corey's dog ate a few cigarettes…..do you think the nicotine in the cigarette could kill him (the dog)? _____

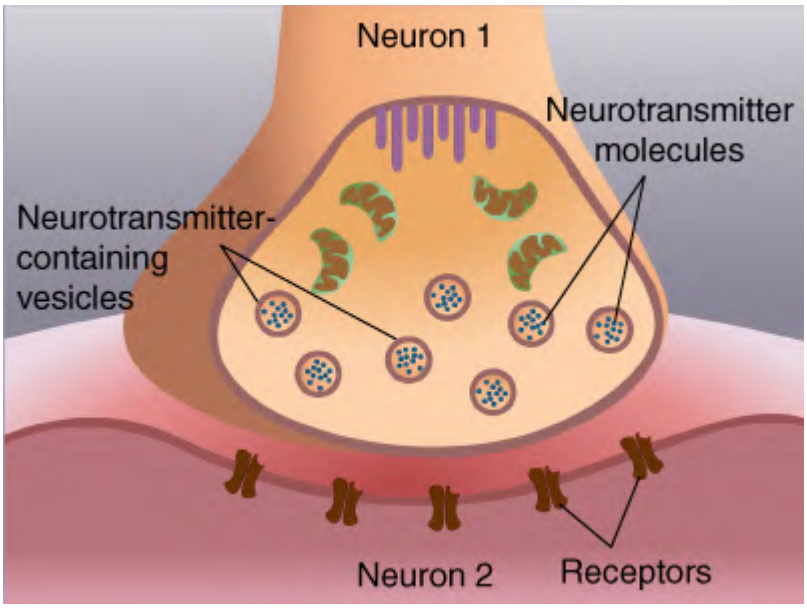
2. Corey's organs are shown below. Draw the path that the nicotine takes after Corey smokes a cigarette to get to the brain. Use arrows to designate the direction.

   Hint: Look at Figure 1.4 (or click the animation) to help you answer this question.



3. In the image below, draw an arrow to the structures at the synapse where the nicotine works.

   Hint: Look at Figures 1.7 and 1.8 (or click on the animations) to help you answer this question.



4. Which of the following effects is Corey likely to feel when nicotine is bound to receptors in his brain? Circle your answer.

   a. moody

   b. anxious

   c. alert

   d. depressed

5. Which of the following effects are produced when nicotine is bound to receptors in Corey's heart and blood vessels? Circle your answer(s).

   a. reduced heart rate

   b. increased heart rate

   c. reduced blood pressure

   d. increased blood pressure

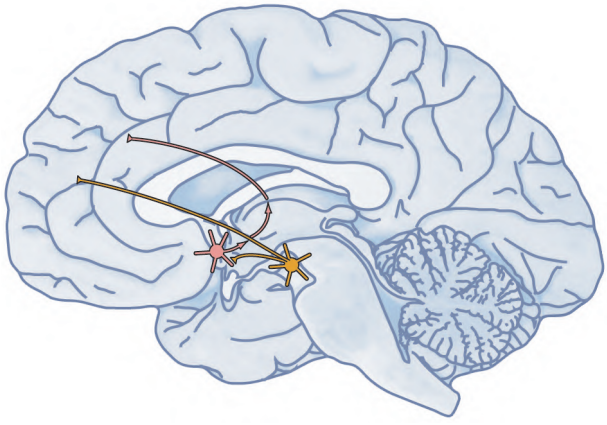
6. What is the main effect of nicotine on neurons?

   a. It increases the neuron's electrical activity

   b. It decreases the neuron's electrical activity

7. When Corey smokes, the nicotine stimulates activity of neurons along the reward pathway in his brain to increase feelings of pleasure (this explains why he likes smoking). Look at the picture of the brain and draw arrows along the reward pathway. Mark where it starts, and where it terminates.

   Hint: Look at Figure 1.9 to help you answer this question.



8. Corey's smoking over time caused several things to happen. Put a number next to the behavior in the order it occurs, and then draw a line to match the term with its definition.

___Addiction	Corey feels normal when he smokes; if he doesn't have a cigarette, he gets moody
___Tolerance	Corey needs more nicotine to get the same effect he got before
___Dependence	Corey can't stop smoking when he wants to; he has lost control over his own decisions

9. Circle the behaviors that are most likely associated with the increased number of nicotine receptors you saw in the smoker's PET scan.

   Tolerance Dependence Addiction

10. Corey started smoking as an adolescent, at a time when the neurons in his brain were still forming their final synapses or connections.

    What are the consequence(s) of this situation? Circle all that apply.

    a. Corey is more likely to become addicted

    b. Corey is more likely to become dependent

    c. Corey is more likely to have trouble quitting smoking

    d. Corey is more likely to try other addictive drugs

Everyone knows cigarette smoking is dangerous and even deadly. We hear about the dangers from our doctors, teachers and parents, and we see warning labels on the packages "May cause cancer". So why do people even smoke cigarettes? Why do approximately 3,000 teenagers begin smoking or using tobacco products each day? And why do over 45 million American adults still smoke, even though they may try quitting many times? There must be a reason why 31 million American adults want to quit smoking, but only about 5% are successful after one year.

The answer lies with a single molecule: **nicotine**. This natural ingredient is found in all tobacco products and can cause intense addiction. With repeated use, tobacco products can also lead to a host of deadly diseases such as cancers of the lung, mouth, and pancreas, **emphysema**, heart disease, and **stroke**. As the noted tobacco researcher M. A. H. Russell once wrote,

> *"There is little doubt that if it were not for the nicotine in tobacco smoke, people would be little more inclined to smoke than they are to blow bubbles or to light sparklers."*

So what is nicotine and how does it lead to addiction? In this module, the following questions are answered:

- What is nicotine?
- How does nicotine enter the body?
- How does nicotine affect the brain?
- Why does nicotine make some people feel good?
- How does repeated use of nicotine products change the brain?
- What are the stages leading to nicotine addiction?
- Are teens more likely to become addicted than adults?

nicotine
The highly addictive chemical found in tobacco products.

emphysema
A lung disease with symptoms that include shortness of breath and difficulty breathing.

stroke
The loss of some or all sensation or movement caused when the brain becomes deprived of oxygen and glucose, due to a blockage or rupture of a brain blood vessel. High blood pressure and heart disease can lead to a stroke.

What is nicotine?

One of the most abundant and highly addictive chemicals found in tobacco products is nicotine. Nicotine is found in high quantities in the tobacco leaves. Each tobacco product contains different amounts of nicotine, but on average, a typical cigarette contains about 10-15 mg, with the average smoker inhaling approximately 1-2 mg of nicotine.

At high doses, nicotine can be extremely poisonous. In fact, nicotine was previously used in insecticides to destroy insects, but its use as an insecticide was banned in the US in 2001 due to contamination of food products. Ingestion of high doses of nicotine causes vomiting, tremors, convulsions, and even death in a short amount of time.

To put this in perspective, just one drop of pure nicotine can kill a person and 60 mg can be deadly. A small child or animal can become very sick or even die from eating just one cigarette left unattended. While ingestion of high doses of nicotine is not very common, the repeated use of products that deliver small amounts of nicotine can lead to a disease called addiction.

smokeless tobacco
Shredded or ground tobacco that can be placed in the nose or mouth. Common forms include chewing tobacco, snus, and snuff. Slang names include rug, plug, chew, spit, and dip.

How does nicotine enter the body?

Depending on the tobacco product used, nicotine can enter the body various ways. Cigarette smokers inhale nicotine from the smoke into their lungs. **Smokeless tobacco** users absorb nicotine through the skin and mucous membranes of the mouth. Nicotine can also enter through the mucous membranes in the nasal cavity (nose) if the oral form of dry snuff is used. Lastly, nicotine can be absorbed through the skin with use of products such as the nicotine patch. Regardless of the means of entry, nicotine quickly enters the bloodstream, where it can then be distributed throughout the body. As smoking is the most common route of entry for nicotine, it will be discussed it in more detail below.

> ***"Very few customers are aware of the effects of nicotine, i.e., its addictive nature and that nicotine is a poison."***
> (1979 tobacco company Brown and Williamson document)

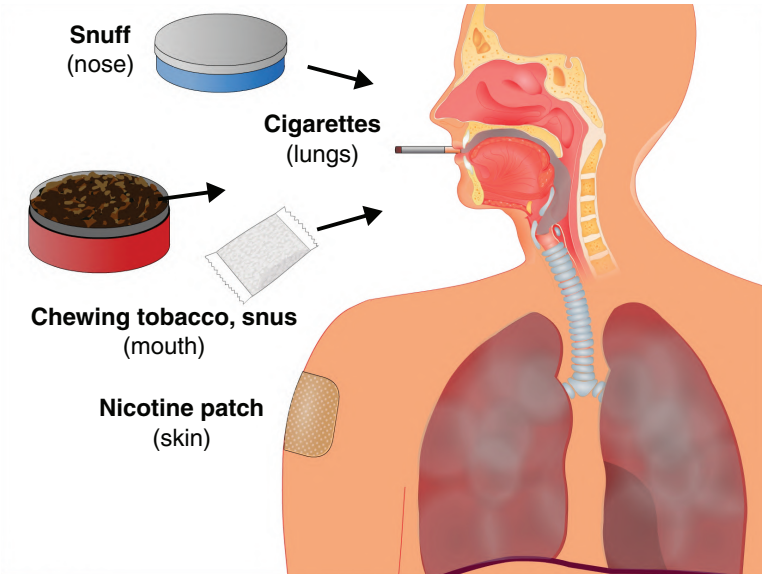


Figure 1.1 Four main routes of entry for nicotine. Regardless of the route of entry, nicotine gets into the bloodstream.

The amount of nicotine a person inhales from a cigarette depends on the smoker's puffing behavior. A person who takes deep and frequent puffs on a cigarette will inhale more nicotine than a person taking short little puffs. On average, a smoker will take about 10 puffs per cigarette to deliver 1-2 mg of nicotine.

alveoli
Tiny air sacs in the lung that participate in the gas exchange of carbon dioxide and oxygen.

Once inhaled, the nicotine travels immediately to the lungs where it reaches millions of tiny air sacs called **alveoli**. There are over 480 million tiny air sacs or alveoli that participate in the air exchange process in the lungs. If all the alveoli were laid side by side they would cover an area the size of a tennis court!

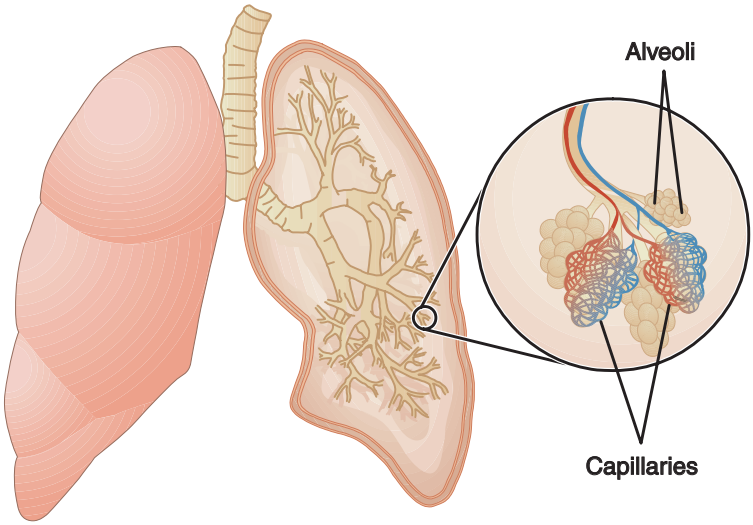


Figure 1.2 The structure of the lungs. Air exchange occurs at the site of the alveoli (tiny air sacs) that are covered with small blood vessels (capillaries).

Each air sac is covered with tiny **capillaries** (blood vessels) through which the blood flows. The large number of alveoli provides ample space (surface area) for oxygen and carbon dioxide exchange. At the site of the alveoli, oxygen, which is breathed in from the air, is absorbed into the bloodstream. At the same time, **carbon dioxide**, which is generated by all cells, travels in the bloodstream to the lungs, where it is exhaled. Likewise, the large surface area of alveoli allows for efficient delivery of nicotine into the bloodstream. Once nicotine enters the bloodstream, it then travels very quickly throughout the body and affects many regions.

capillaries
The smallest type of blood vessel found in the body.

carbon dioxide (CO_2)
Chemical gas composed of one carbon molecule and two oxygen atoms. It is produced by all cells in the body as a waste product, and is released in the breath during exhalation.

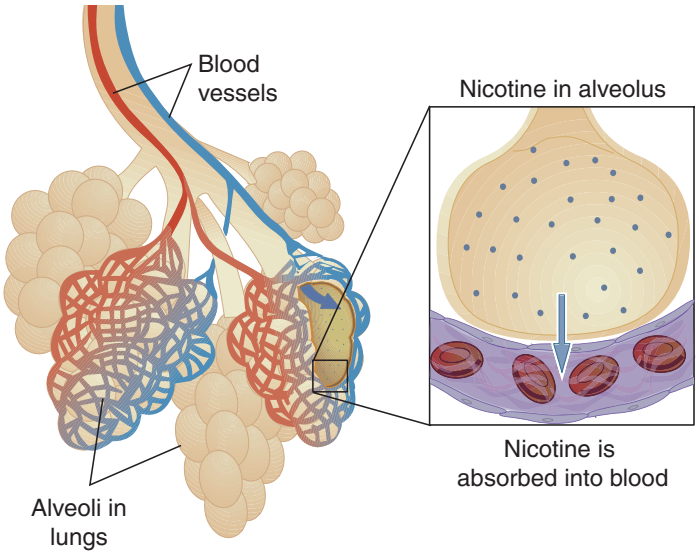


Figure 1.3 Nicotine absorption into the bloodstream from the lungs. The huge surface area of the alveoli is a very efficient way to get nicotine into the bloodstream.

How does nicotine affect the brain?

One of the first areas to which nicotine travels is the **brain**. Regardless how nicotine enters the body (inhalation, mouth, nose, or skin), it will be absorbed into the bloodstream and reach the brain quickly. The fastest way to get nicotine to the brain is to inhale it—it only takes

brain
The organ enclosed in the skull that carries out all cognitive and mental functions

about 10 seconds for nicotine to reach the brain. This is because the blood from the **lungs** travels to the **heart**, which then pumps the blood through the **arteries** to all major organs, including the brain.

lung
The organ in the body responsible for gas exchange.

heart
The organ responsible for pumping blood throughout the body.

artery
Blood vessel that carries blood from the heart to all organs in the body.

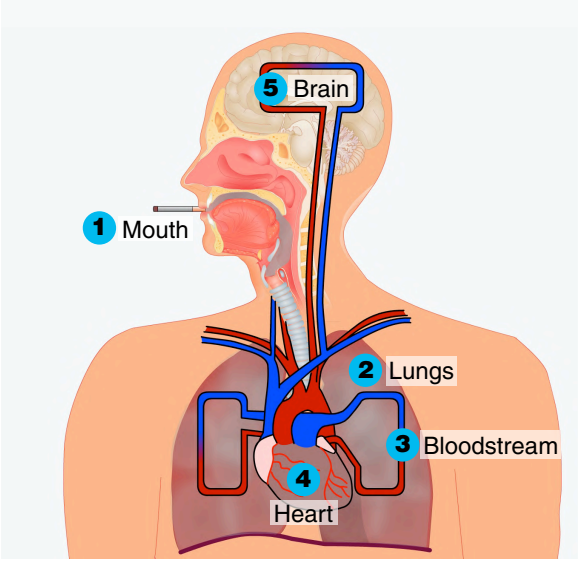


Figure 1.4 Path that nicotine travels from the mouth to the brain. Nicotine is inhaled from cigarette smoke and travels directly to the lungs, where it is immediately absorbed into the bloodstream. Once in the blood, nicotine travels to the heart, where it is then pumped throughout the entire body, including the brain.

Click on the picture to see an animation.

The brain is the master control center

Once nicotine reaches the brain, it triggers a series of responses that alters the way cells in the brain communicate with each other. A brief review the function of the brain will help in understanding how nicotine causes its effects.

The brain is an amazing part of the body—it's composed of billions of tiny cells called **neurons**, which communicate with each other to control all functions throughout the body. The brain regulates all aspects of life – pumping of the heart, breathing, walking, emotions, and memories. The brain is what differentiates humans from all other types of living species—humans are the only species to rationalize situations, have wild imaginations, and perform high level thoughts and tasks.

neuron
A nerve cell that communicates with other cells using electrical and chemical signals.

How do neurons communicate with each other?

One of the primary effects of nicotine is to alter the way that neurons (i.e., brain cells) communicate. Neurons communicate with each other through both electrical and chemical signals. Each neuron consists of a **cell body** (the main part of the neuron that contains the nucleus, or the

cell body
The main part of the neuron (major cells in the brain) that contains the nucleus, in which the genetic material is found.

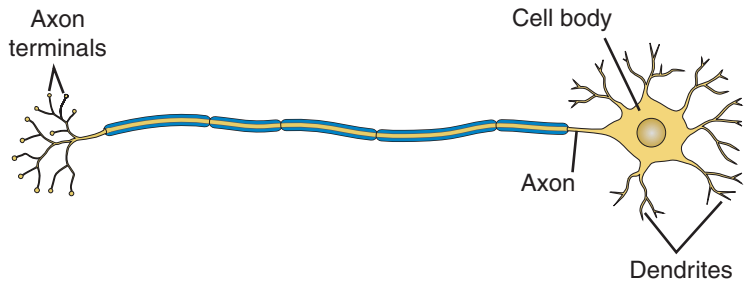


Figure 1.5 Neuron structure. Each neuron consists of a cell body attached to one long axon and several shorter dendrites.

cell's control center), an **axon**, and many **dendrites**. The axon is one long extension from the cell body that carries electrical signals from one end of the neuron to the other end (referred to as the terminal). These signals travel extremely fast--up to hundreds of miles per second. Dendrites are shorter extensions that also branch off the cell body. Think of the dendrites as tree roots that branch out. The dendrites receive signals from other neurons in the form of chemicals.

axon
A long extension from the cell body of a neuron that carries electrical signals toward the synapse.

dendrite
Short branch-like extensions of the neuron that receive chemical signals from other neurons.

Neurons communicate information to each other when they are in close contact. The connections between neurons are called **synapses**. On average, one neuron can form about 1,000 synapses with other neurons. With millions of neurons forming thousands of neural connections, it is estimated that the number of neuron-to-neuron connections in the brain exceeds the number of stars in our galaxy.

synapse
The connection between two neurons; it is where most communication between neurons occurs.

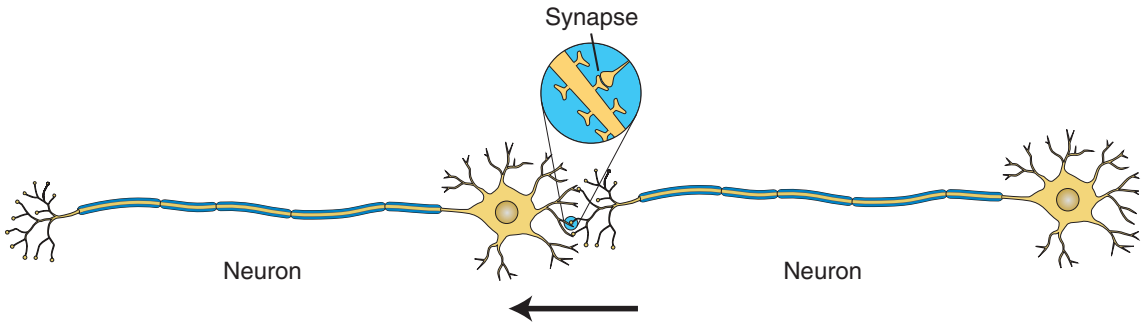


Figure 1.6 The synapse—the connection between two neurons. The synapse is the location where communication between neurons occurs. The arrow shows the direction in which information (i.e., electrical activity) flows.

So how does the communication work at the synapse? First, an electrical impulse travels down the axon toward the terminal. Once there, the electrical signal triggers the release of chemicals called **neurotransmitters** from tiny sacs into the synaptic space outside the terminal. These chemicals actually carry the "messages" from one neuron to another. Once in the synaptic space, the neurotransmitter attaches to specific proteins called **receptors** on the dendrites of the

neurotransmitter
A chemical released from one neuron and binds to receptors on another ("chemical messenger").

receptor
A protein to which hormones, neurotransmitters, and drugs bind. Receptors are usually located on cell membranes and elicit a response once bound.

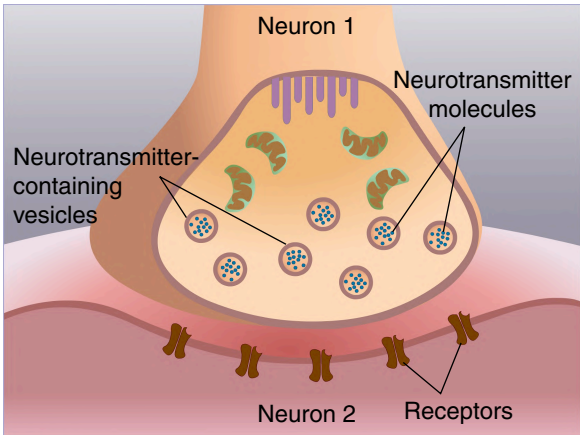


Figure 1.7 Neurons communicate at the synapse. Neurotransmitters are released from vesicles in the terminal to the outside of the neuron. The neurotransmitters then bind to receptors on a neighboring neuron's dendrites, causing a cellular response (a change in electrical activity).

Click on the figure to see an animation.

neighboring neuron. There are thousands of receptors on the dendrites, with each type of receptor recognizing only a very specific neurotransmitter, similar to a lock and key. When the neurotransmitter binds (or attaches) to a specific receptor, the receptor will either increase or decrease the electrical activity of the neuron on which it resides.

acetylcholine
A major neurotransmitter in the brain that provides signals for a variety of actions, including level of alertness, learning, and memory. Also located in neurons outside the brain, it provides signals to the heart, blood vessels, and other muscles to contract.

Let's consider one of the major neurotransmitters in the brain called **acetylcholine**. Interestingly, nicotine mimics this neurotransmitter. Nicotine acts just like acetylcholine, but works even better. This is described below. Acetylcholine binds to receptors ("acetylcholine receptors") that increase the electrical activity of neurons, resulting in more signals transmitted to neighboring neurons. Once acetylcholine has done its job, it is destroyed. New acetylcholine must be made by the cells to repeat the whole process.

Nicotine increases electrical activity in neurons to cause several effects

nicotinic receptor
A protein that binds both acetylcholine and nicotine to increase the electrical activity of neurons.

As previously mentioned, nicotine mimics the effect of the neurotransmitter acetylcholine. When nicotine enters the brain, it can actually bind to the same receptors that bind acetylcholine. For this reason, the acetylcholine receptor is often referred to as a **nicotinic receptor**.

However, when one uses a tobacco product or another nicotine-containing product, there is more nicotine available in the synaptic spaces compared to acetylcholine. The nicotine competes with acetylcholine to bind to the nicotinic receptors and it wins. Now, with more nicotinic receptors activated by nicotine, a more intense response is produced.

So while acetylcholine normally provides the just the right amount of alertness when it binds to its receptors, nicotine produces a much more intense response (increased alertness, pleasurable feelings) due to its higher concentrations at the acetylcholine receptors.

Now you can review the entire process of how neurons communicate and the effect of nicotine. Watch the 3D animation on a video clip.

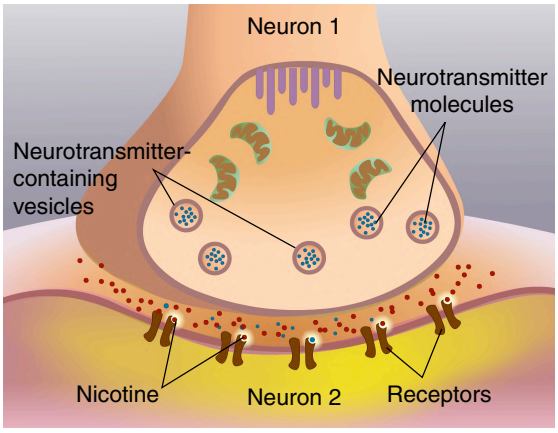


Figure 1.8 Nicotine competes with acetylcholine to increase electrical activity in neurons. Since there is more nicotine in the synaptic space, it wins, and produces larger electrical currents, and heightened responses.

Click on the figure to see an animation.

Nicotinic receptors are found in many other regions of the body besides the brain. Not surprisingly, nicotine acts in the body wherever nicotinic receptors are found, such as on the heart, blood vessels, and muscles. The widespread distribution of nicotinic receptors explains why nicotine will increase heart rate, blood pressure, and muscle contractions.

Why does nicotine make some people feel good?

Dopamine and pleasure feelings

When some people use a product containing nicotine, they feel good. Others do not—so they do not continue to use the product. But for those individuals who do like the effect of nicotine, they will continue to smoke or use nicotine-containing products. So what causes the feeling of pleasure in some tobacco users?

There is a group of neurons that forms a pathway located in the middle of the brain called the "reward pathway" or the "pleasure pathway." The reward pathway is activated by natural rewards like food and water. Activation of the pathway by natural rewards gives us the feeling of wanting more, a necessary function to ensure our survival as a species. But drugs are even better activators of the pathway.

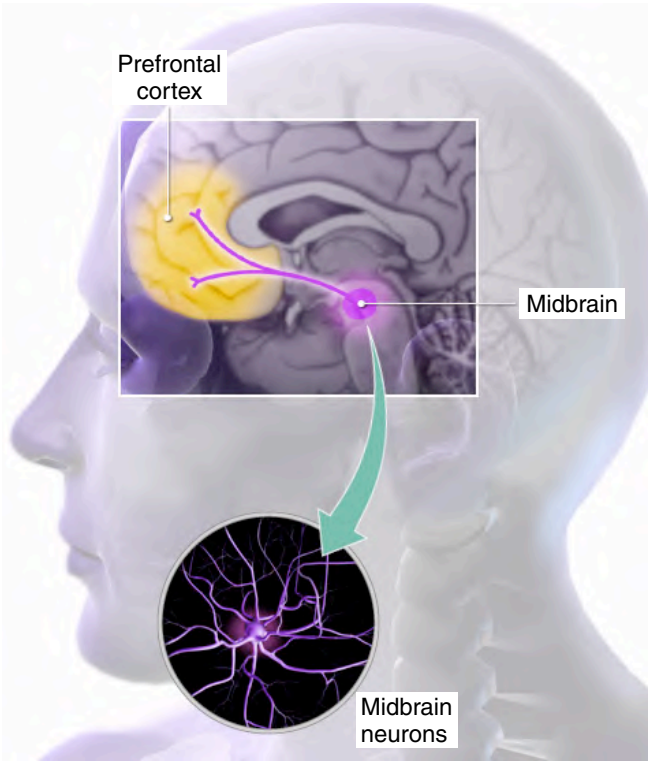


Figure 1.9 The reward pathway. The reward pathway begins in the midbrain where cell bodies of neurons send long fibers to the prefrontal cortex. Think of the midbrain cell bodies like airline hubs whose airline routes (neuron fibers) extend to the coastline (pre-frontal cortex). Nicotine and other addictive drugs act on receptors in this pathway that activate rewarding feelings.

For example, nicotine activates the pathway by triggering the release of another neurotransmitter called **dopamine**. Dopamine has been termed the "pleasure neurotransmitter" because it causes pleasurable feelings when it binds to its receptors ("dopamine receptors") in the reward pathway.

dopamine
A neurotransmitter that is involved in the brain's response to emotions, movement, and ability to respond to feelings of pleasure. The pleasurable response to addictive drugs results from stimulation of dopamine action within the brain's reward pathway.

Once activated by nicotine, the reward pathway transmits information that we perceive as feelings of pleasure, and just feeling really good. Nicotine acts in other parts of the brain to reduce anxiety and stress.

Scientists have discovered that this same reward pathway is also activated by drugs like alcohol, cocaine, and heroin. This discovery explains why nicotine and other drugs that produce rewarding feelings can be a first step toward addiction.

However, nicotine effects are short lived because the drug is quickly metabolized (broken down) in the body. Thus, when nicotine is no longer present, the pleasurable experience decreases and nicotine users feel the need for more nicotine in the body just to function normally, so they smoke another cigarette.

withdrawal
Negative side effects felt by a habitual drug or tobacco user when the substance is no longer present in the body. Nicotine withdrawal symptoms include irritability, hand tremors, anxiousness, inability to sleep, and depressed mood.

dependence
The user (or organism) functions normally only in the presence of the drug; removal of the drug results in negative symptoms (withdrawal). Dependence develops with repeated use of the drug.

addiction
The compulsive use of a drug, despite the experience of negative consequences—no control over use.

tolerance
Results when a person (or organism) requires a higher dose to get the same effect; or, the same dose no longer produces the same level of effect that was present initially.

How does repeated use of nicotine products change the brain?

Repeated nicotine alters the brain structure

When one continues to use a product with nicotine in it, a strange thing happens. The number of nicotinic receptors—that is, acetylcholine receptors—increases on the neurons! With more receptors present, the person needs more acetylcholine binding to them to feel normal. But the neurons can only make so much acetylcholine. So what is the result? The person needs more nicotine to feel normal.

When the nicotine is not present, a person will often get symptoms such as headaches, tremors, shakiness, and an overall feeling of irritability and frustration. To get rid of these "**withdrawal**" symptoms, the user will smoke another cigarette or use another tobacco product and begin to feel much better. The presence of withdrawal symptoms is typical of **dependence** and it almost always precedes **addiction**.

In addition, the increased nicotinic receptors can also explain **tolerance**, or the need to use more of the product containing nicotine to get the original effect. The distinction among tolerance, dependence, and addiction is explained in detail below.

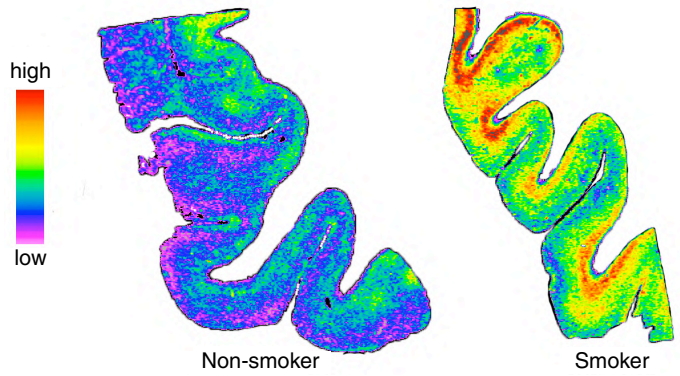


Figure 1.10 Nicotine causes changes in the brain. A section of the front of the brain is shown from a non-smoker and a smoker. The number of nicotinic receptors (in yellow and red) is significantly increased in the smoker compared to the non-smoker. (From Perry et al., 1999)

The good news is that the increase in receptor number is probably not permanent. Once one stops using a product containing nicotine, the number of receptors will return to normal pre-nicotine levels—although

this could take more than a year. However, immediately after stopping smoking for example, many users experience unpleasant withdrawal symptoms because the brain now has an excess of nicotinic receptors, which are unoccupied. The long process for the brain to recover to normal is one reason why many people who try to quit using nicotine products can't get past the first year (or even week!) nicotine-free.

What are the stages leading to nicotine addiction?

When a person uses a drug with rewarding properties (like nicotine, alcohol, or cocaine), they are at risk for addiction. Although they don't become addicted immediately, there are 2 stages that usually precede addiction—these include tolerance and dependence. Let's review the sequence in the context of smoking cigarettes (although it's the same for any tobacco product).

Stage 1: Tolerance

After smoking only a few cigarettes, nicotine loses its effectiveness at stimulating the reward pathway. Scientists have shown that the nicotinic receptors can lose their sensitivity to nicotine. Now, the person must smoke more cigarettes because more nicotine is necessary to cause the same effect. This smoker has become tolerant to nicotine, which usually happens first, even before dependence sets in.

Stage 2: Dependence

As the smoker continues to smoke, the smoker becomes dependent on nicotine. Dependence means that the smoker requires nicotine just to function normally. So, if a dependent smoker were to stop smoking, he/she will experience withdrawal. The symptoms of nicotine withdrawal can range from mild to severe and include anxiousness, inability to sleep, and depressed mood. Withdrawal can begin within as little as a few hours after the last cigarette. Without thinking about it, people smoke enough cigarettes or use enough tobacco products (i.e., chewing tobacco, etc.) each day to keep the levels of nicotine in their brain at a relatively constant level and to feel the effects all day long.

Stage 3: Addiction

compulsion
An intense urge to perform an action, with little control to stop the action.

Once the smoker shows nicotine tolerance and dependence, addiction follows. Addiction to nicotine occurs when a person becomes a **compulsive** tobacco user and can no longer control how often he/she uses tobacco—regardless of the negative consequences. Addiction can creep up quickly (some recent evidence suggests addiction could begin as soon as 2–3 weeks from use of the first cigarette), or it could occur over a longer period. The user is not really aware it is happening, but there are some symptoms.

The most obvious symptoms include:

- the person can't control when he/she smokes a cigarette or uses tobacco products (compulsive use)
- constant thoughts about getting a cigarette or other type of tobacco product
- craving that results when he/she can't get nicotine that's contained in the product

Often people who are addicted to nicotine think that they can control their smoking, and can quit any time. However, if they quit, withdrawal symptoms emerge first and then craving results. Craving (and stress!) almost always leads the person to relapse, and he/she starts smoking again. The person who starts smoking by age 19 typically takes about 20 years to finally quit.

"The desire to quit seems to come earlier now than before, even prior to the end of high school…attempts to quit are very painful, they thought they could quit easily, but they soon learn…"they have become SLAVES to their cigarettes. (Imperial Tobacco)

Sequence of stages leading to addiction

- *Tolerance occurs when smokers require more nicotine just to have the same effect they felt initially. It usually precedes dependence.*
- *Dependence occurs when smokers require nicotine just to function normally*
- *Tolerance and dependence lead to nicotine addiction—one can't quit.*

Are teens more likely to become addicted than adults?

Brain changes during development

For years, people have noticed a remarkable difference between a child's or a teenager's decision-making ability and that of an adult. However, only recently scientists discovered a biological explanation for this difference. The brain continues to develop and mature until about the mid-20's, with the most dramatic changes occurring during adolescence. The major changes happen during the teen years in the part of the brain called **prefrontal cortex**, which is responsible for functions such as self-control, judgment, thinking, emotions, and decision-making.

prefrontal cortex
The frontal region of the brain involved in thinking, impulsive and compulsive behavior, and decision making. It is one of the last areas of the brain to develop.

Because the prefrontal cortex is still maturing, teenagers are more likely to make poor decisions such as trying drugs or alcohol without fully comprehending the long-term consequences. You might remember that the prefrontal cortex is the place in the brain that the reward pathway terminates (see Figure 1.9).

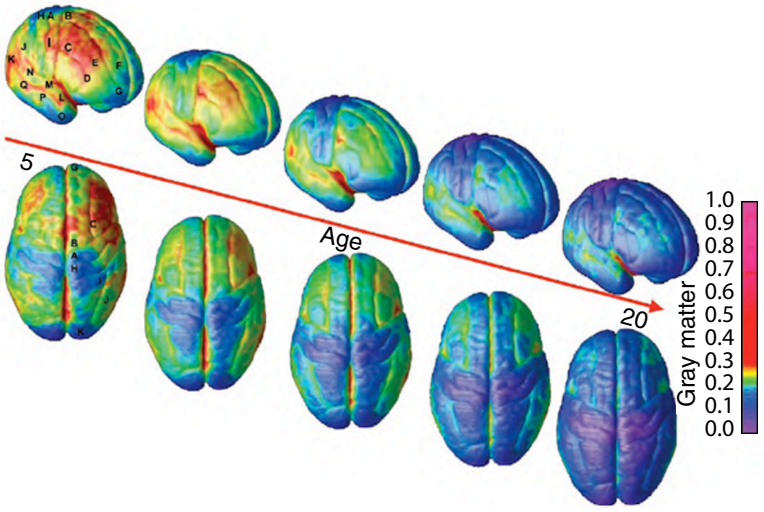


Figure 1.11 Human brain development. Brain images are shown from age 5 (left side of picture through age 20 (right side of picture). The darker red colors show regions of the brain that are still growing and maturing, with blue regions indicating regions that are fully mature. (*Image courtesy of Gogtay, with permission.*)

The human brain is not fully mature until the mid-20's, thus leaving teenagers more vulnerable to developing a nicotine addiction.

The teenage brain is extremely vulnerable to nicotine and other addicting drugs

The teenage brain is particularly vulnerable to drugs such as nicotine and alcohol. These drugs can have much more dramatic consequences on the developing brain than in an adult brain. How do we know this?

Here's what we know about smoking and human brains: Both adults and teen smokers show increased nicotinic receptors in their brains compared to non-smokers. Scientists use a type of brain scan called a **PET scan** to look for receptors in the brain. A typical PET scan of nicotinic receptors in human brains shows the difference between smokers and non-smokers (adults or teens).

PET scan
Technique used to visualize and measure blood flow, cellular activity, or receptor density in the brain.

Even more information about adolescent brains can be learned from animals. Many animals have a similar brain structure to humans and serve as an excellent model to study human diseases. For example, scientists gave nicotine (by injections) to adolescent rats and found the same increased number of nicotinic receptors in rat brains as seen in human brains. But, they were able to show something even more interesting…the increase in nicotinic receptors in the adolescent rat brain occurred more rapidly compared to the adult rat brain, and the young brains did not recover to normal as easily.

So the animal studies provide a clue as to what occurs in humans. It is difficult to do these kinds of studies in adolescent humans, so we must rely on data from the rats. Nevertheless, it is clear that the adolescent brain structure changed after exposure to nicotine. The nicotine-induced

increase in the amount of nicotinic receptors at this early age suggests that adolescents can become addicted to nicotine fairly easily.

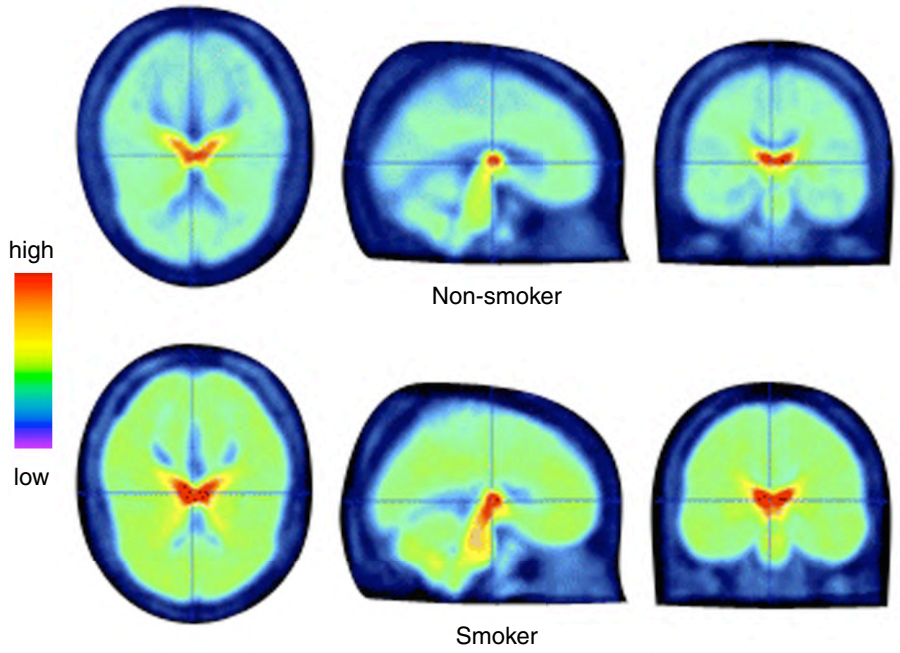


Figure 1.12 PET images of nicotinic receptors in the human brain. The increased number of nicotinic receptors (red and yellow) in the smoker's brain is dramatic. Top view (left), side view (middle), front view (right). (*Image courtesy of Mukhin, with permission*).

Reasons why teens become addicted to nicotine more easily than adults:

- The region of brain that controls judgment and decision-making is not completely developed in teens
- The increase in the number of nicotinic receptors in adolescent brains occurs more rapidly, and persists, compared to adults. More receptors lead to the need for more nicotine.

Teen smoking and addiction statistics

The following lists national trends for smoking behavior in teenagers (reported as of 2007).

- *90% smoking adults begin smoking by age 21, one half of adult smokers are addicted before their 18th birthday.*
- *Every day, 4,000 children between ages 12-17 smoke their first cigarette.*
- *1,300 of those smokers will become addicted.*
- *Half of those will die from smoking.*
- *If smoking trends remain the same, over 6.4 million current teen smokers will die prematurely from smoking related causes.*
- *Smoking rates are declining in teenagers.*
- *Teen smoking is associated with many other unhealthy activities, such as carrying weapons, trying different drugs, fighting and engaging in high-risk sexual activity.*
- *Smoking teens are three times more likely to drink alcohol, eight times more likely to smoke marijuana, and 22 times more likely to try cocaine.*
- *Teens are more likely to develop a severe addiction to tobacco than if they begin smoking later in life.*
- *50% of smoking teenagers have unsuccessfully tried to quit, failing to break the addiction.*

511,527 kids became new smokers in 2008

170,509 of those kids will die early because of their addiction

The Role of Tobacco in the Development of Cancer

Content in this module will help students gain a scientific understanding of the how cancer develops, the cell's protective measures to defend against cancer, and the mechanisms by which tobacco constituents weaken these protective capabilities.

The specific concepts covered in this module include:

- How cancer develops
- Specific mechanisms by which tobacco contributes to cancer development
- Introduction to DNA, genes, and proteins
- Description of the main carcinogens found in tobacco
- Genetic variations and how they contribute to changes in protein function
- Cell's defense mechanisms to protect against cancer
- Interaction of genetics and environment that contribute to cancer development

Learning objectives:

After completing this module, it is expected that students will be able to:

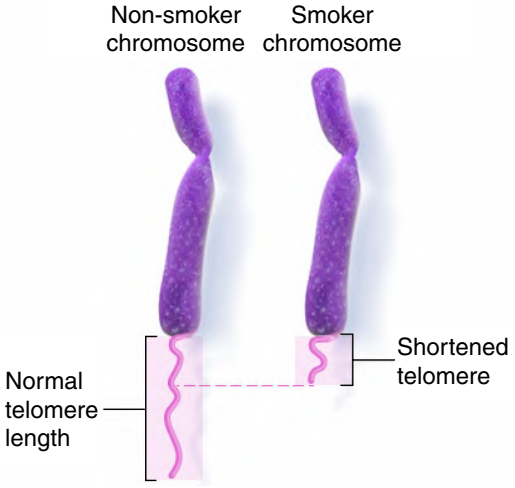
- Explain basic cell terminology, including DNA, gene, and protein
- Describe the two major types of carcinogens in tobacco
- List the steps involved in cancer formation and tumor growth
- Discuss the damaging consequences of carcinogens to the DNA
- Describe the protective mechanisms of the cell after exposure to tobacco
- Discuss the short and long term effects of carcinogens in the cell
- Describe the roles of genetics and the environment in cancer development

Story #2

Can Tobacco Cause Mutations in my Genes?

About two years ago, Corey got some bad news. His mom developed lung cancer. He was really surprised because his mom didn't smoke cigarettes. Corey's mom explained to him that she used to be a smoker when she was a teenager but quit when she married his father. She explained that quitting was very difficult and it took her several years to completely give up smoking, but that she was very glad she had finally succeeded. Before she was diagnosed with lung cancer she participated in a medical study interested in determining if smoking could cause structural changes in one's chromosomes, increasing the risk of cancer. The scientists were interested in former smokers too, and offered to pay $500 to subjects to enter the study. Of course, Corey's mom signed up.

The researchers took a blood sample and examined the chromosomes in her white blood cells. (The chromosomes look the same in every cell in the body). They compared them with chromosomes from non-smokers and with people who were former or current smokers. Here's what they found:



The non-smoker had a chromosome with a long tail called a "telomere" at the end of it. Corey's mom, had a shortened telomere, just like the smoker. The researchers told her that it was likely that she would get cancer, although they weren't sure which kind. Unfortunately, they were right.

Corey's mom knew that Corey had been smoking, and she told him, "I know you don't think this can happen to you, but given your genetics, and your smoking habit now, you are at pretty high risk for getting cancer too. So you have the choice to do something about it now". Do you agree with her?

Your second "mission" is to figure out Corey's risk of getting cancer. You will become an expert about chemicals in tobacco that cause tumor development and cancer. You'll even get to participate in an experiment. Consult the Module 2 guide to answer the questions below for Mission #2.

Name _____ Group #_____

Mission #2

Answer the following questions:

1. Tobacco contains many carcinogens (chemicals that cause cancer). Two of the major carcinogens in tobacco that cause cancer in humans are BaP and NNK. Match the 2 carcinogens with the way in which they are formed in the tobacco.

   BaP (benzo[a]pyrene) NNK

   When tobacco is "cured" or prepared for either smokeless or smoked forms, this chemical is produced from nicotine in the tobacco: _____.

   As tobacco is burned, this chemical is produced as part of the combustion process: _____.

2. It only takes one carcinogen to produce one mutation that can cause cancer. Name one gene that is mutated by carcinogens such as BaP leading to the development of cancer.

   _____

   What is the function of the protein that is synthesized under instructions of this gene?

3. Nicotine, in the absence of any tobacco, can also help tumors form. How can this happen? Circle your answer(s).

   a. Nicotine causes DNA damage

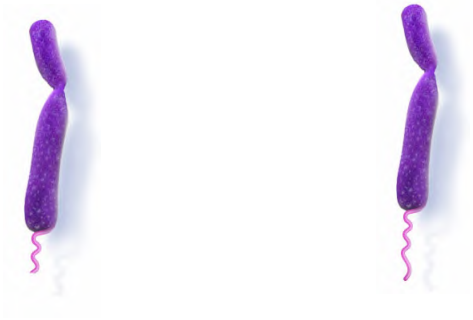
   b. Nicotine prevents chemotherapy drugs from killing cancer cells

   c. Nicotine causes tumor growth

   d. All of the above

4. Suppose people could get "telomere tested" when they go for their annual health checkup. They would give a cheek swab sample, and the lab would check the telomere length of their chromosomes. Can you match which chromosome is most likely found for each of the people below? (draw an arrow to connect the telomere with the people)

Young person **Smoker with cancer** **Old person**



5. It usually takes years to develop cancer after smoking or using smokeless tobacco. But how long does it take to form a mutation in DNA, which is one of the first steps in developing cancer? One way to find out is to participate in a real experiment. You can do this experiment in a laboratory or follow along with a video of other students performing the experiment in a lab.

   Before you start, indicate what you think the answers are to the following questions by checking the item:

   a. Can exposure to tobacco cause a mutation in DNA without smoking it?

      Yes____ No____

   b. How long would it take for tobacco to cause a mutation?

      Hours____ Days____ Months____ Years____

   Now, consult your lab packet to do the experiment—you can watch the video and then collect your own data. When you have finished, answer the following questions:

   a. Could the tobacco cause a mutation in DNA without smoking it?

      Yes____ No___

   b. How long did it take for tobacco to cause a mutation?

      Hours____ Days____ Months____ Years____

   c. Do your answers agree or disagree with your prediction above?

      Agree_____ Disagree_____

d. Now that you have determined how tobacco from one cigarette can cause a change in bacterial DNA, how could you test whether nicotine alone could cause a mutation in DNA? Use the lab activity you just saw in the video or in your Lab Activity packet to help guide your answer.

The warnings are posted in magazines, newspapers, and on cigarette packages: Smoking Causes Lung Cancer, Heart Disease, Emphysema, and May Complicate Pregnancy. But how do these things happen? What actually causes the **cancer**? What about chewing tobacco, cigars, and other forms of tobacco—do they cause cancer too? What is the chance that someone will actually develop cancer if (s)he smokes or uses other forms of tobacco products? Why do some people develop cancer while others don't?

cancer
Abnormal and uncontrolled growth of cells in the body.

This next section will explore how the toxic chemicals in tobacco can lead to cancer. The following topics will be explored:

- What is cancer?
- What type of tobacco products cause cancer?
- What compounds in tobacco products cause cancer?
- How do carcinogens in tobacco products actually cause cancer?
- What about nicotine—can it cause cancer?
- Why do most cancers take so long to develop?
- How does genetics play a role in tobacco and cancer?

What is cancer?

Cancer is defined as an abnormal growth of cells in the body that leads

tumor
Uncontrolled growth of cells that leads to the formation of a large mass of tissue.

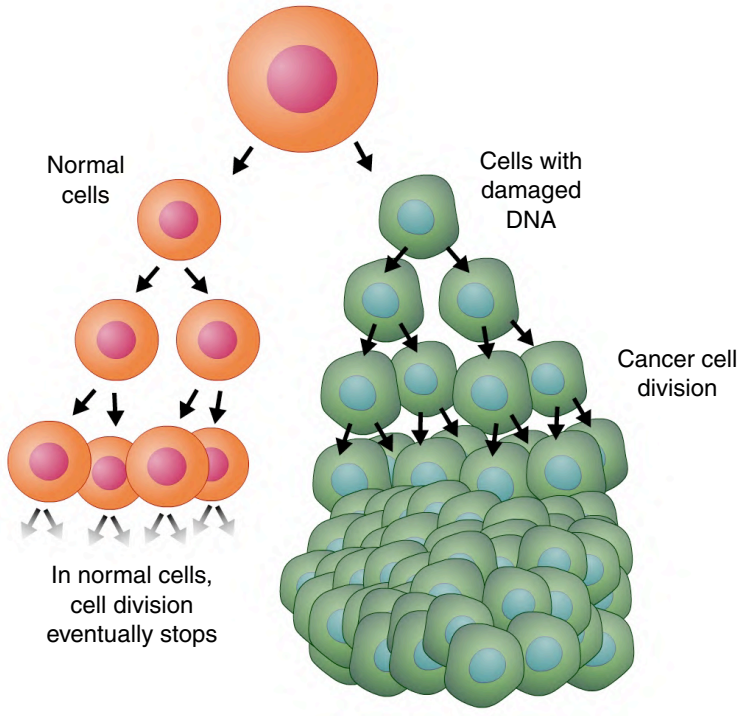


Figure 2.1 Cancer cell division. One cell has defective or damaged DNA. This causes it to divide more quickly than healthy cells, resulting in large numbers of cancer cells that form a tumor.

to the formation of a large mass of tissue, called a **tumor**. Normally, all cells in the body undergo a process of growth and division at some point in time. However, cancer begins when something happens inside the cell that causes them to divide uncontrollably.

Healthy cells contain specific proteins that signal the cell to stop dividing and growing when no longer necessary. But sometimes, the "stop" signals get corrupted, leading to uncontrolled cell growth and division. The disruption of the stop signals can be caused by genetic, or inherited, defects in the cell's **DNA**, or by external dangers such as radiation or environmental toxins, such as pollutants or chemicals, both of which can cause damage to the cell's DNA. Each of these factors can lead to cancer.

DNA
A large molecule of heredity material that contains the genetic information; DNA, or deoxyribonucleic acid, is found in the nucleus of every cell.

How does cancer spread?

Early in the development of cancer, the abnormal growth of cells begins in one particular location in the body (usually where the cells with DNA damage are located) and produces a tumor. As the tumor grows, tumor cells can break away and enter the bloodstream (**metastasis**), where they are then free to travel to any part of the body and continue their rapid and uncontrolled cell growth. People with metastatic cancer often have a lower chance of survival because the cancer has already spread throughout the body. Once cancer begins infecting other regions of the body, it becomes very difficult to completely destroy all of the cancer cells.

metastasis
Movement of cancer cells away from the primary tumor site to different locations within the body.

As cancer cells reproduce, they begin to deprive normal healthy cells of nutrients, minerals, and chemicals that are essential to their survival. If the normal cells can't survive, then the function of the organ to which they belong declines, which can lead to illness in the patient. Unfortunately, for many cancer patients, the cancer cells can eventually overtake the normal cells in the body causing death in people who would otherwise still have many years of life left.

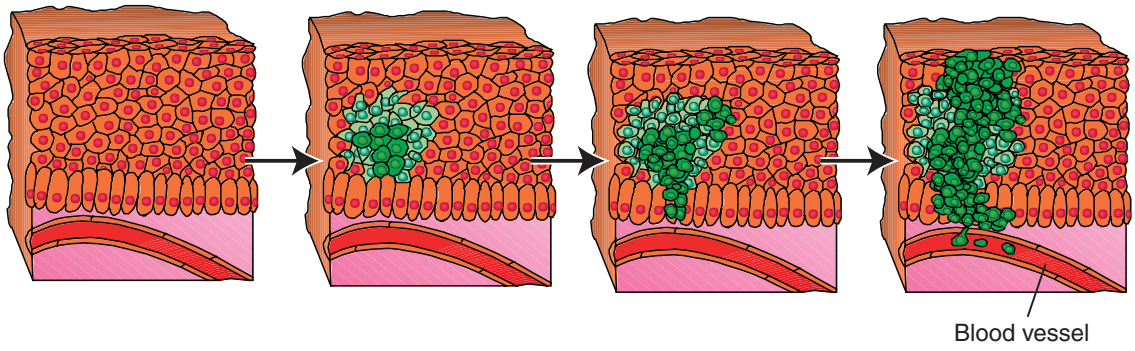


Figure 2.2 Tumor growth and metastasis. Cancer cells (green) lead to the formation of a tumor in the tissue of an organ. Some cancer cells can break away from the tumor and enter the bloodstream. Once in the bloodstream, the cancer cells can travel to other areas of the body, causing new tumors to grow (metastasis).

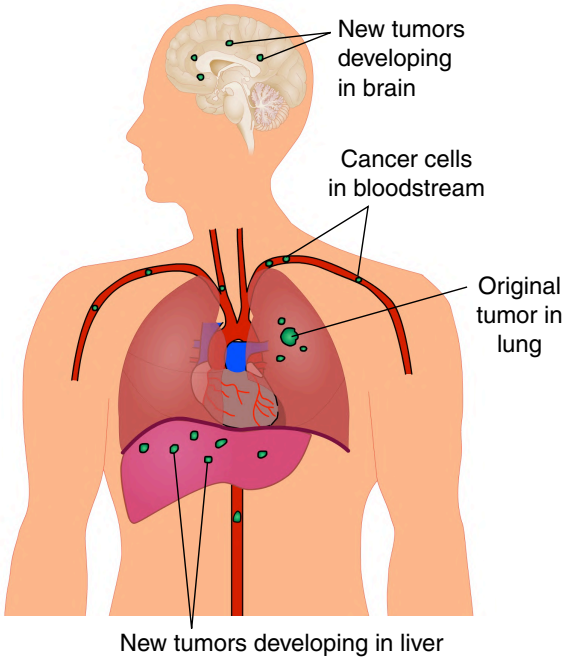


Figure 2.3 Cancer cells spread to the entire body. Once cancer cells enter the bloodstream, they can travel to any region of the body (metastasis). As the cancer cells enter other organs in the body, new tumors may develop.

Use of tobacco products causes many kinds of cancer

Use of tobacco products is one of the most common risk factors for many types of cancers, including cancer of the lung, pancreas, mouth, throat, bladder, liver, stomach, colon, and nose to name a few. Tobacco use is estimated to cause about 30% of all cancer deaths worldwide.

The most common and widely publicized type of cancer from cigarette smoke is lung cancer, which is caused primarily by the inhaled smoke. The risk of lung cancer dramatically increases with the number of cigarettes smoked. Lung cancer is one of the most preventable cancers—it is estimated that 90% of lung cancers are directly related to

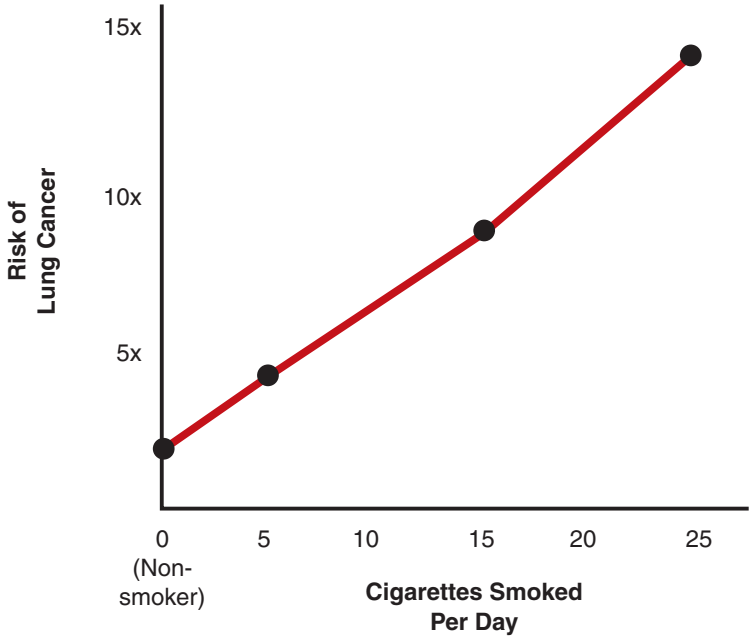


Figure 2.4 Lung cancer risk increases with cigarette use. The graph shows that the risk of lung cancer increases as one increases the number of cigarettes smoked per day. Someone who smokes 5 cigarettes a day has twice the risk of lung cancer compared to a non-smoker, although even a non-smoker has a small risk of lung cancer. [Data from "Understanding Cancer", National Cancer Institute]

tobacco products (including both smoke inhaled by the smoker and smoke inhaled by someone in close proximity to the smoker, called **second-hand smoke)**. Because lung cancer is such a deadly disease, millions of lives worldwide could be saved each year if tobacco products were decreased or even eliminated.

second-hand smoke
Refers to the tobacco smoke inhaled by someone in close proximity to the smoker without actually smoking the tobacco product (cigarette, pipe, cigar, etc.)

Although tobacco use in the form of cigarette smoking is declining in countries such as the USA, many other countries are experiencing an increase in tobacco use, especially within the poorer countries. In 2008, the **World Health Organization** predicted that within 2 years, cancer deaths could become the most common form of death in developing countries such as China and India, primarily due to the increased use of tobacco products in these countries. Not only does tobacco contribute to the development of cancer, it also leads to many other types of illnesses, such as addiction, heart disease, stroke, and lung diseases.

World Health Organization
A world-wide organization that is the leading authority in making health decisions that affect countries throughout the world.

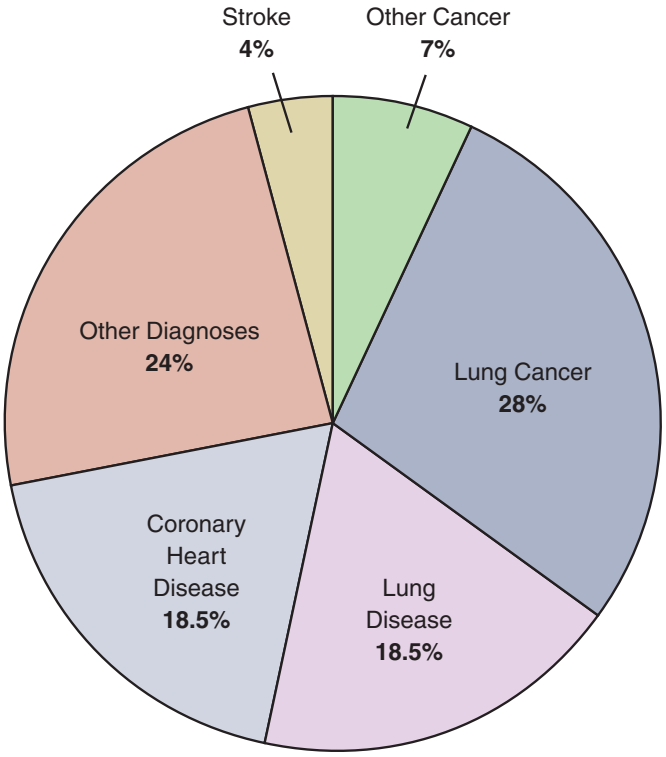


Figure 2.5 Types of diseases caused by cigarette smoking and death rates in the U.S. About 440,000 people each year in the U.S. die from smoking-related diseases. The largest percentage of deaths is due to lung cancer. [Data from "Understanding Cancer", National Cancer Institute]

Every eight seconds, someone dies from tobacco use

What compounds in tobacco products cause cancer?

All tobacco products contain a toxic mixture of dangerous chemicals. For example, just one cigarette contains over 4,800 chemicals, many of which are deadly in large quantities or are known to cause cancer. Chemicals used to make rat poison, arsenic (a type of poison), insecticide, nail polish remover, car batteries, and nuclear weapons are just some of the toxic chemicals found in tobacco products. In fact, these chemicals are found not only in cigarettes, but in all types of tobacco products, including chewing tobacco, pipes, snuff, cigars, and tobacco lozenges.

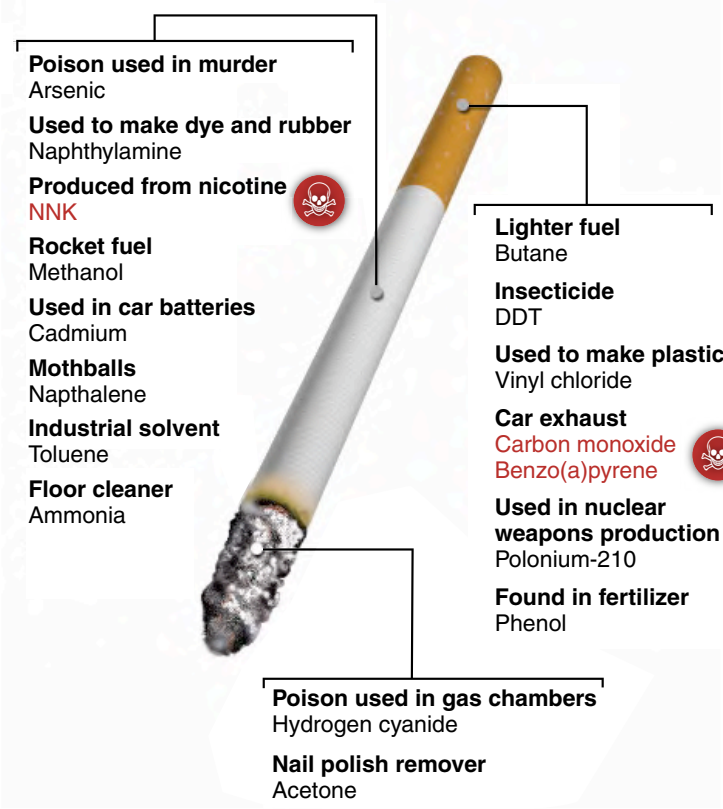


Figure 2.6 Toxic chemicals found in cigarettes. This a partial list of some of the toxic chemicals and poisons found in a cigarette and cigarette smoke.

The very fine particles in cigarette smoke (often referred to as "**tar**") contain many harmful ingredients, including 69 known **carcinogens**. A carcinogen is a chemical that is known to cause cancer in animals and humans. Most likely, many more carcinogens exist in tobacco than have been identified. Every carcinogen in tobacco has the ability to cause cancer independently from another. Furthermore, the chance of developing cancer increases each time someone is exposed to carcinogens (e.g., each time a cigarette is smoked, or smokeless tobacco is used).

Although not all carcinogens in tobacco products have been yet identified, there are some that are considered the major carcinogens. These include two chemicals—one is called **benzo[a]pyrene**, or BaP, and the other is a **nitrosamine** called **NNK**.

tar
The mixture of toxic chemicals found in the cigarette smoke.

carcinogens
Any chemical that is known to cause cancer in animals and humans.

benzo[a]pyrene (BaP)
A carcinogen found in tobacco smoke that is produced by the burning of cigarettes; it is found in many combustion reactions such as the burning of fuel.

nitrosamine
Chemical compounds found in tobacco products that are highly carcinogenic; two well known examples include NNK and NNN.

NNK
A carcinogen found specifically in tobacco that belongs to a family of chemicals called nitrosamines. NNK is produced during the curing (or preservation) process of tobacco used in cigarettes, smokeless tobacco, and other products.

BaP is produced by combustion, such as the burning of a cigarette, or the burning of fuel, like that found in automobile or jet plane exhaust. When a cigarette is lit, the burning of the tobacco produces large amounts of BaP in the cigarette smoke. Once in the body, BaP binds directly to DNA within the cell and causes **mutations** (changes to the DNA structure). Often times, the DNA mutations can generate "instructions" leading to uncontrolled cell growth and eventual tumor formation.

mutation
Change in the DNA sequence caused by environmental or genetic factors.

Although BaP can bind to DNA in any type of cell, it has a tendency to bind more often to DNA in lung cells than other types of cells. We'll explore all about mutations and cell growth in more detail below.

The other major carcinogen in tobacco is NNK. NNK is produced during the curing (or preservation) process of tobacco. NNK is found in most forms of tobacco, including cigarettes and smokeless tobacco. Scientists have shown that NNK exposure leads to cancer of the lung and the pancreas.

Major Carcinogens in Tobacco

BaP

NNK

Similar to BaP, NNK binds to DNA in many types of cells and causes DNA mutations. These mutations may lead to the development and growth of cancers. Although NNK can bind to DNA in any type of cell, studies have found that NNK may bind more often to DNA in lung, pancreas, and mouth cells.

Pancreatic cancer is one of worst cancers to develop, as it often carries a death sentence in as little as 5 months. Once it's detected, very few people survive pancreatic cancer after just 2 years.

NEWS FLASH!! New research has found that nicotine may also have the ability to cause tumor growth. For more discussion about nicotine, see below.

How do carcinogens in tobacco products actually cause cancer?

Compounds in tobacco products cause cancer in several ways. Basically, they promote the development of mutations, or changes in the structure of DNA, causing damage to cells and triggering uncontrollable cell growth and division. In order to understand how this happens, it is useful to review briefly how damage to DNA can cause cancer.

A brief review of DNA

DNA (deoxyribonucleic acid) is the building block of our **genes** and **chromosomes**. DNA is found in every cell within the body and it carries the recipe for building all **proteins** that cells use to function properly.

DNA is made up of 4 molecules (also known as **nucleotides** or DNA bases). There are over 3 billion of these nucleotides in each cell, and they are packaged to form 23 pairs of chromosomes that reside in the cell's nucleus. The DNA nucleotides are arranged in a very specific order that contain the "recipe" for each protein to be made.

Think of nucleotides like letters in the alphabet. When letters are arranged in a certain way, they form words that make sense to a reader. This is the same concept for DNA. The nucleotides are arranged in an order that makes sense to the cell. The "words", which are composed of strings of DNA nucleotides, are called genes. Each gene instructs the synthesis of a particular protein in the cell, with a specific function. So if the order of the nucleotides is changed, then the function of the gene could change, leading to a problem with the resulting protein.

Every cell's DNA in a person is identical. And surprisingly, each person's DNA is 99.9% identical to every other human in this world, regardless of their nationality or ethnicity. This 0.1% sequence difference in each person is what makes each person unique.

genes
Segments of DNA that carry the complete genetic information (instructions) to make a protein.

chromosome
Compact structure of DNA and proteins located in the nucleus of a cell. Every cell in the human body contains 23 pairs of chromosomes.

protein
A functional molecule formed by specific instructions contained within the DNA. There are approximately 30,000 different proteins made from DNA; each protein has a unique function in the body.

nucleotides
Building blocks of DNA. DNA is composed of strings of 4 nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C).

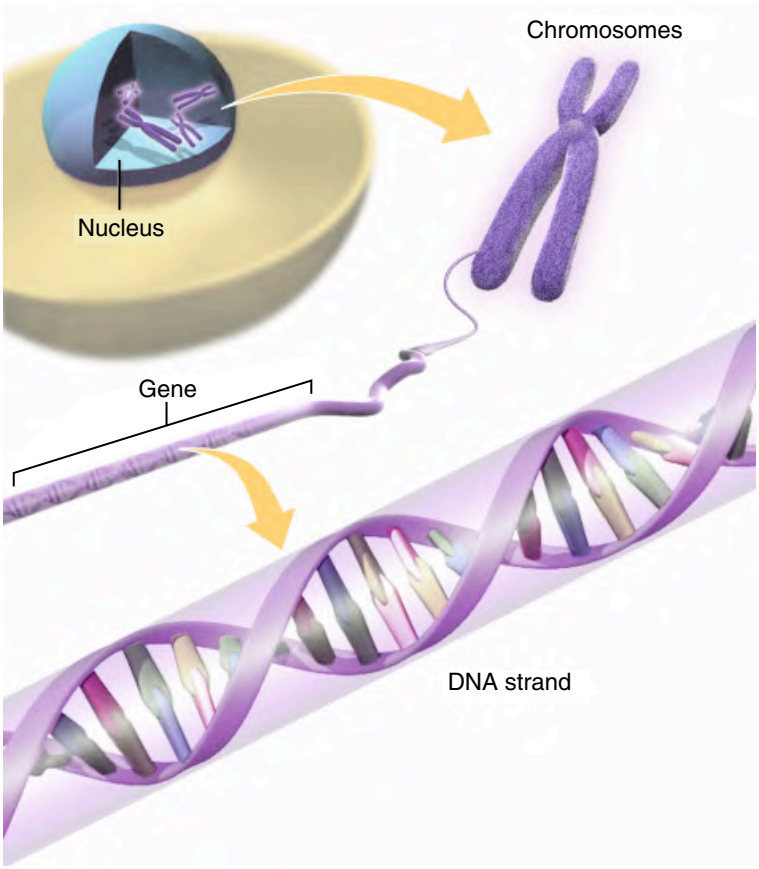


Figure 2.7 Strands of DNA form genes. DNA is found in every cell in the body. In the cell nucleus, twisted strands of DNA make up the chromosomes. A segment of DNA called a gene instructs the synthesis of specific protein. Proteins do all the work in a cell.

Many cells in the body are constantly dividing and replicating (or reproducing an exact copy). Each time a cell replicates, the DNA replicates as well. Replication of DNA is a critical process of cell replication. Because DNA carries the recipe for making all protein components of the cell, damage to the cell's DNA could change the entire function of the cell and may also cause problems in cell replication. For example, a cell containing damaged DNA could be replicated indefinitely, producing new copies with damaged DNA.

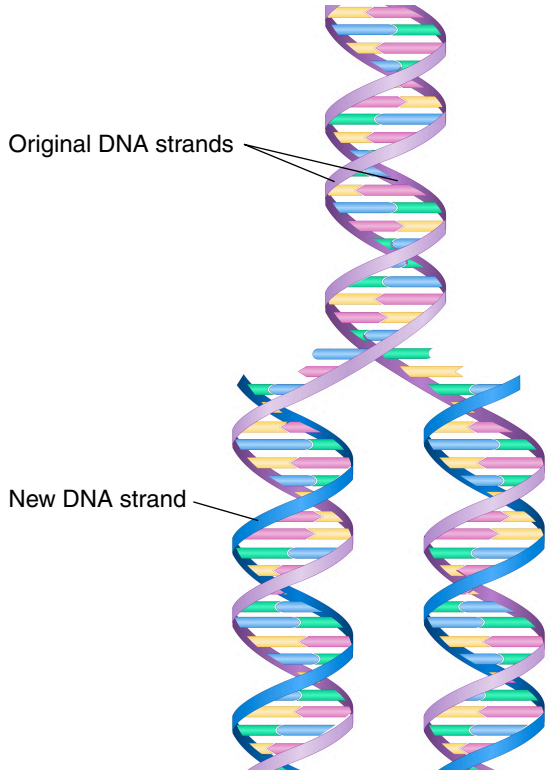


Figure 2.8 DNA replication. Each of the original strands of DNA is replicated to form an identical copy. One copy of the DNA is found in the newly created cell nucleus and one copy remains in the original cell nucleus.

Cells protect "themselves" from damage to their DNA

The cell has many mechanisms to ensure that each DNA molecule replicates to form an identical copy. In addition, cells contain certain proteins ("spell-checkers" and "proof-readers") that make sure that there are no "spelling" errors, or mutations in the nucleotide sequence before DNA replication.

Sometimes a mistake happens (meaning a mutation does occur) and the cells produce proteins that "instruct" the cell to actually commit suicide. This form of cell death is called **apoptosis**, and it is often the cell's last resort for making sure that the DNA mutations do not get replicated.

The **p53** protein is an example of a protein that instructs cells to undergo apoptosis when spelling errors or mutations in the DNA occur. So p53 comes to the defense--it promotes apoptosis of cells with damaged DNA, thereby eliminating cells that might go on to develop into a cancer.

apoptosis
A form of cell death that is triggered by a series of signals originating in the cell's own genetic instructions ("cell suicide"). Apoptosis is the cell's mechanism to destroy itself when there is an error or mutation in its DNA.

p53 protein
A protein that provides signals to the cell to undergo apoptosis ("suicide") when it has mutations in its DNA. It is one of the major proteins that suppresses the formation of tumors.

Despite the help from a "protective" protein like p53, problems can arise. For example, sometimes a mutation can occur in the region of DNA that is responsible for making the "spell-checker" or "proof-reader" proteins. In fact, mutations can occur in the p53 gene. Remember that one of the jobs of the p53 protein is to instruct cells to die when mutations are present. So if the p53 gene now has a mutation, the p53 protein can't do its job to get rid of cells with mutations. Instead, the cells with damaged DNA keep dividing and produce copies of the mutated DNA in the newborn cells. The cycle goes on to produce cancer.

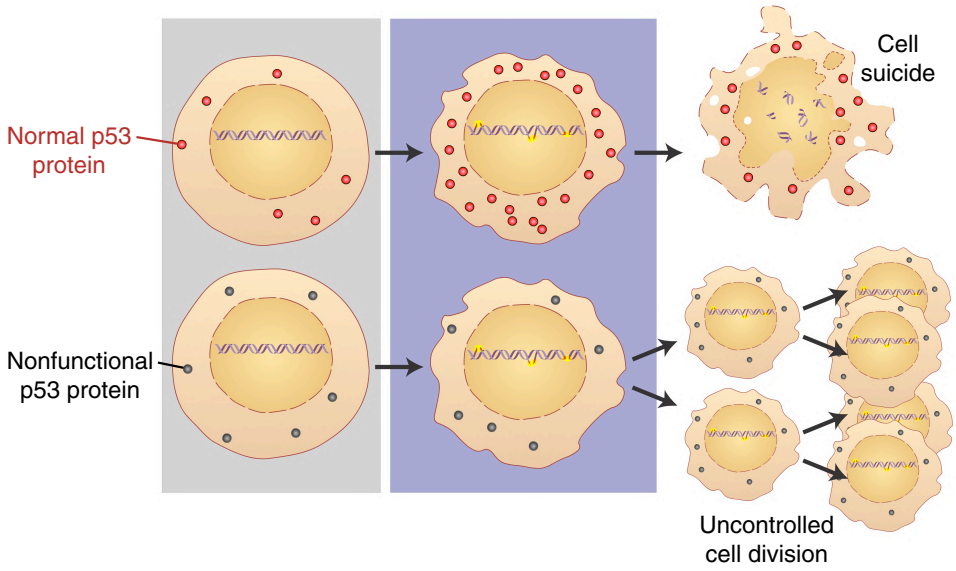


Figure 2.9 The p53 protein directs suicide of cells with DNA damage. Normally the p53 protein helps cells destroy themselves ("suicide") when their DNA becomes damaged (top row). This prevents the cell from going on to replicate, generating cancer cells. But if the gene for p53 has a mutation, then the p53 protein doesn't work properly to help the cell commit suicide. Instead, the cells with damaged DNA go on to divide uncontrollably, leading to the development of tumors (bottom row).

Carcinogens damage DNA

Certain chemicals can damage the cell's DNA and its ability to replicate correctly. These chemicals typically include carcinogens, many of which are found in tobacco products. Carcinogens can bind to DNA to cause mutations. If the mutations occur in genes that normally protect the cell from DNA damage, then the cell begins to replicate, producing more copies of the damaged DNA.

The BaP found in tobacco smoke is a good example of a carcinogen that binds to a segment of DNA for the p53 gene—this is the one that directs cell suicide, or apoptosis. The binding of BaP to the p53 gene causes a mutation in the gene, which can result in the formation of a tumor. Mutations in the p53 gene are found in about 50% of all human cancers, including approximately 60% of lung cancers. Even if BaP was

the only carcinogen in smoke, smokers would still have a high risk of developing cancer.

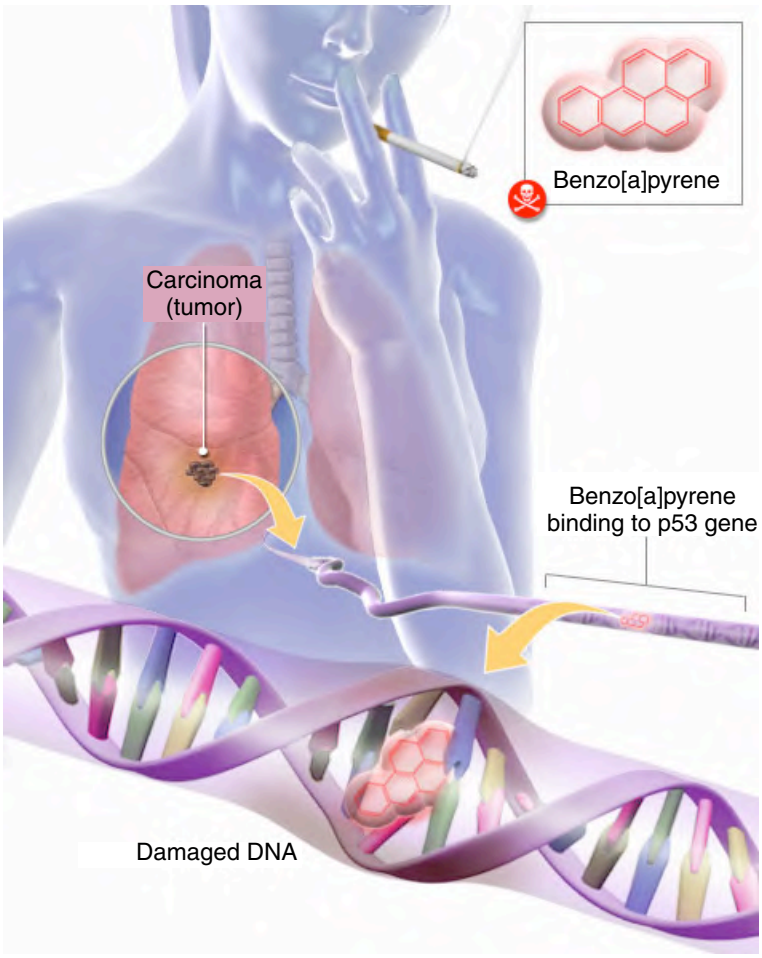


Figure 2.10 Benzo[a]pyrene (BaP) causes lung cancer. Carcinogens from cigarette smoke such as BaP bind to DNA in lung cells. Benzo[a]pyrene binds to the p53 gene segment of DNA, causing a mutation in the p53 gene that leads to lung cancer.

Smokers can damage their DNA tips (telomeres)

Carcinogens in tobacco smoke can cause additional problems for DNA—at the tips. There is a small strand of DNA at the tip of each chromosome called a **telomere**. Think of the telomere as the plastic cap on the end of a shoe-lace (chromosome) that keeps it from fraying with wear.

telomeres
Small strands of DNA at the ends of the chromosomes that protect the DNA from damage during cell division. The telomeres shorten with each cell division, and if they become too short, the DNA is no longer protected.

The telomere protects the DNA from damage each time the cell divides, but in the process of every cell division the telomere gets a bit shorter. Once the telomeres reach a certain length, the cells stop dividing, and the DNA remains protected. But if the telomeres get too short, the DNA is no longer protected, thus putting it at increased risk of getting mutations.

The unprotected DNA is another factor that promotes the development of cancer. Additionally, the length of the telomere region may also tell us something about an individual's health. Healthy young people have long telomeres, but as people age the telomeres get shorter.

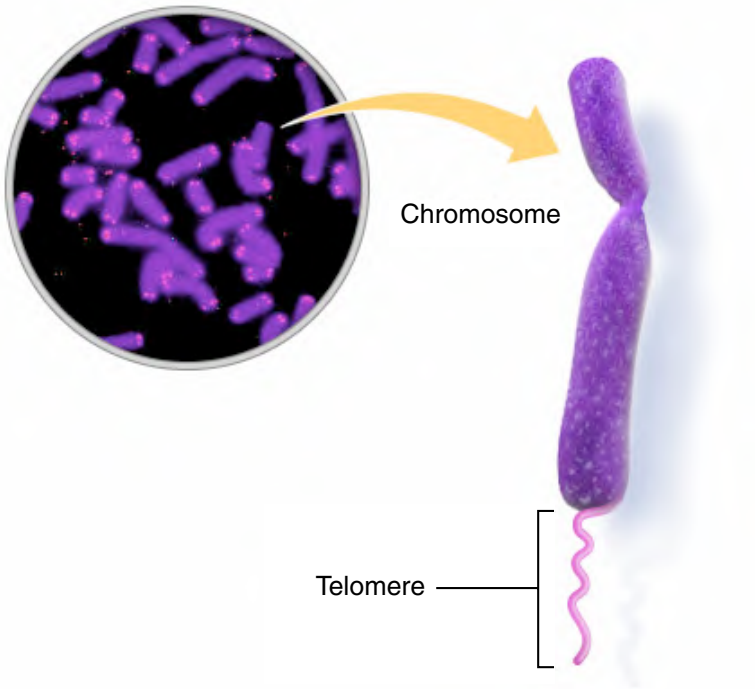


Figure 2.11 Telomeres are located on the ends of the chromosomes. These "caps" of DNA protect the DNA from damage each time the cell divides. They get a little shorter with each cell division.

So, what does this have to do with smoking? Scientists have recently discovered that smoking cigarettes causes telomeres to shorten prematurely. While it has always been obvious that smoking cigarettes results in certain signs of premature aging such as wrinkled skin, with new technologies, scientists have found that smokers have shorter telomeres than non-smokers of the same age. Scientists estimate that

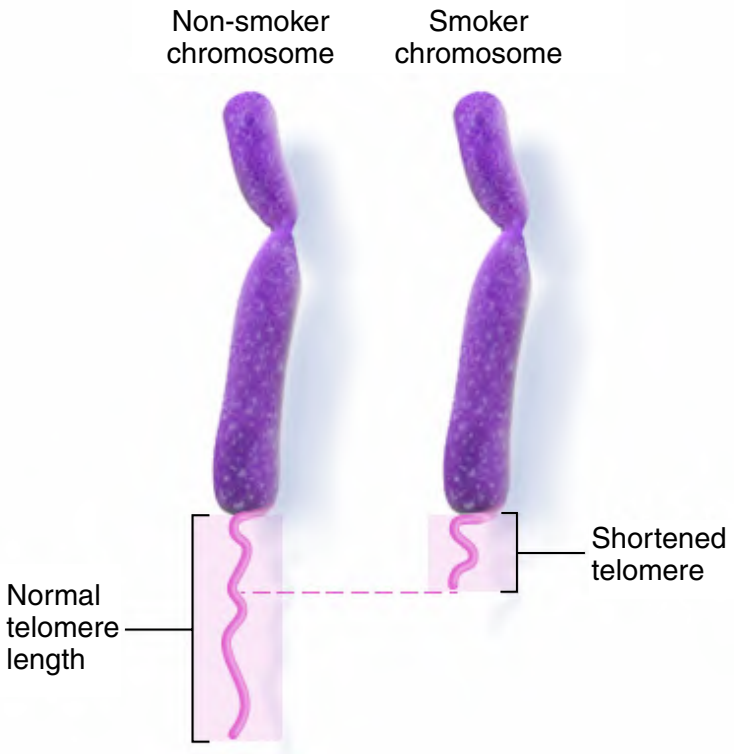


Figure 2.12 Smokers have shortened telomeres. Contrast the chromosomes of a non-smoker (left) and a smoker (right). The shorter telomere of the smoker's chromosome reflects premature aging of about 5 years, and increases the risk of cancer.

the length of the smoker's telomere would be approximately the same length of a non-smoker who is almost 5 years older.

Because of shortened telomeres, smokers have an even greater risk of developing cancer and other diseases. Shortened telomeres could explain, in part, why smokers typically die younger than non-smokers of other age-related diseases, even if they do not get cancer.

National studies have determined that when smoking is started in teenage years and continues into adulthood, the typical smoker's life span is approximately 8–10 years less than the non-smoker.

Smokers have shorter telomeres, which may result in a shorter lifespan than a non-smoker

Carcinogens cause cancer by:

- ***Binding to DNA and producing mutations.***
- ***Shortening the telomeres, putting the DNA at increased risk to develop mutations. It only takes one carcinogen and one mutation to cause cancer.***

What about nicotine? Can it cause cancer?

For many years, scientists have debated whether or not nicotine causes cancer. The problem is that no one was actually using nicotine as a drug by itself (without the tobacco) over an extended time period, so there was no way to tell if it was causing cancer in people.

But now scientists have shown that nicotine can cause DNA damage (to cells in the mouth for example) and may help tumors grow larger, especially lung and colon tumors. In addition, nicotine seems to "undo" the benefits of chemotherapy. Chemotherapy is a type of cancer treatment consisting of drugs that kill rapidly dividing cells. Nicotine prevents drugs that are used to treat cancer from killing the lung cancer cells, although scientists are still trying to determine how this happens.

It has been known for a long time that people who undergo chemotherapy to treat their cancer have a poor survival rate if they continue to smoke during the treatment. For patients who are addicted, they might stop smoking during chemotherapy, but decide instead to use a smokeless tobacco product or even a nicotine patch. By doing this, they may be preventing the cancer treatment from working, and suffer the same fate as if they continued to smoke.

Now that nicotine-only products are on the market, it will take a few decades to see if the rate of cancer development in users is similar to that of smokers or oral tobacco users.

Role of Nicotine in Cancer

- *May damage DNA*
- *May contribute to tumor growth*
- *Prevents chemotherapy drugs from killing cancer cells*

Why do most cancers take so long to develop?

Most cancers take years to develop and often occur in people as they get older. This long process is mainly due to the cell's protective mechanisms to keep cancer from developing. However, as cells age, the chance of accumulating harmful mutations increases and cancer cells can start to grow. Once the cells become cancerous, it can take years of continuous dividing for the cancer cells to produce a human tumor that is large enough to cause illness or migrate to other tissues.

However, the long "incubation" period doesn't mean that nothing is happening. Every time a tobacco user is exposed to a carcinogen, there is the potential for a mutation to develop in their DNA.

As discussed above, the cell's protective mechanisms try to get rid of cells with damaged DNA. But as more mutations occur in the DNA, the higher the chance that some of the damaged cells will escape this fate, and then replicate the mutated DNA.

Thus starts the long road to cancer. So although a person who starts to smoke in the teen years may not show any evidence of the disease until much later in life, the teen still has a high risk of accumulating mutations in his/her cellular DNA with each cigarette puff or use of a tobacco product. The more mutations that go undetected in the cells, the higher the chance the damaged cells could lead to cancer.

The earlier a person quits using tobacco, the less likely the person will get cancer later in life. Unfortunately the cancer risk never goes back to pre-smoking levels. But as the body remains free of carcinogens, the cells have more time to repair the damage to their DNA. Thus, tobacco users who quit are at a decreased risk of developing cancer and other tobacco-related diseases compared to those who continue to use tobacco products for the remainder of their life.

How does genetics play a role in tobacco and cancer?

Scientists have known for hundreds of years that certain traits tend to run in families. Children often share similar features with their parents (such as hair color, eye color, height, etc.). It is also well known that certain diseases occur in several members within the same family. But it wasn't until the 1950's that researchers discovered how and why traits and diseases run in families. The answer lies in the discovery of DNA.

DNA is the hereditary material in every cell in the body that contains all of the information passed on from parent to child. As researchers learn more about DNA, it is becoming clearer that almost all diseases are due to slight structural changes in DNA. These changes or differences in DNA sequences (variations) between individuals make them more or less likely to develop certain diseases.

Genetics of nicotine addiction and cancer

So what does genetics have to do with smoking? Is the desire to smoke inherited from our parents? Why are some people able to give up tobacco products quickly and while others try to break their habit for their entire lives? What about cancer – why do some people get lung cancer after smoking their entire lives while others do not?

As discussed above, one may inherit a piece of DNA that increases her/his risk of cancer or addiction. In fact, scientists have recently discovered that genetics can play a strong role in nicotine addiction.

Studies have shown that people with certain variations in specific segments of their DNA are more likely to begin smoking, become addicted to nicotine quicker, have a harder time quitting using nicotine products, or are more likely to develop cancer.

> *Genetic differences can explain why some people may develop cancer or become addicted much quicker to nicotine than others.*

Surprisingly, one of the variations in DNA sequence associated with these problems has been located at the gene that makes the nicotinic acetylcholine receptors. As discussed previously, these receptors bind the nicotine, resulting in the cascade of events that leads to nicotine addiction.

The environment is a key factor

However, genetics is not the complete story. For example, just because someone has an altered gene sequence that puts them at increased risk for developing cancer doesn't mean that the person will get cancer. And, a difference in the gene sequence for the nicotinic acetylcholine receptor

in certain individuals doesn't force someone to take their first puff on a cigarette.

More than likely, it is the environment that someone lives in that determines if (s)he will decide to begin smoking (or using other tobacco products). In this case, the environment refers to peer pressure by friends and family, living with relatives that smoke, exposure to tobacco advertisements, etc.

Thus people may begin smoking based on the environment they live in, but once they start, if they have certain variations in their DNA, it may make it even harder for those people to give up smoking.

Genetic factors and the environment work together to determine one's overall chance of developing a disease.

Gene tests for addiction and cancer

Currently, there are no genetic tests to determine accurately if one will develop cancer or become addicted to nicotine. These diseases probably result from differences in many genes, not just one gene. So testing for one gene may not be the best predictor.

However, research is just beginning and future findings will likely produce a much clearer picture of how our environment interacts with our DNA. Thus, although scientists are closer to identifying which individuals are at risk of cancer and addiction based on their genetic profile, it is the combination of genetics and how the individual interacts with his/her environment that will be the best predictor of whether the person develops an addiction to nicotine and/or develops cancer.

Tobacco Products in Today's Market

Content in this module will help students gain a scientific understanding of the health consequences of using alternative tobacco products, such as reduced-nicotine/tar cigarettes, chewing tobacco, snus, and nicotine-only products.

The specific concepts covered in this module include:

- Differences between regular and tar/nicotine-reduced cigarettes
- Carcinogens in alternative tobacco products
- Addiction risk using alternative tobacco products
- The use of nicotine replacement therapy for quitting

Learning objectives:

After completing this module, it is expected that students will be able to:

- Discuss the risks of addiction and cancer following use of regular and reduced-nicotine/tar cigarettes
- Explain why reduced-nicotine and tar cigarettes are just as harmful as regular cigarettes
- Name and describe the harmful effects of four types of alternative tobacco products
- Discuss the addiction potential of alternative (both smoked and smokeless) tobacco products
- Discuss the pros and cons of nicotine replacement therapy products

Story #3

Am I Free of Risk with Alternative Tobacco Products?

After hearing the news about his mom, Corey decided to try to quit smoking now. However, quitting was much harder than he thought. Each time he tried to quit, in the first few hours, Corey kept shaking and couldn't concentrate. Sometimes he got very grumpy and irritable. When he smoked a cigarette, these symptoms went away. One day at school, Corey's friend Michelle said, "Hey, you could try one of those new cigarettes that doesn't have as much nicotine or carcinogens, so you won't be as addicted and you won't be as likely to get cancer like your mom". So Corey took Michelle's advice and switched from his regulars to a reduced-nicotine cigarette.

Unfortunately for Corey, he kept smoking, and actually smoked more of the reduced-nicotine cigarettes compared to his regulars. Michelle pointed this out to him, and apologized, saying, "Gee, I had no idea that would happen. Why don't you try using smokeless tobacco? At least you won't be breathing in the smoke, that's got to be better for you." So again, Corey took her advice. He did switch to using smokeless tobacco and continued to use it for several years, but couldn't quit the smokeless stuff either. The problem was that now he was receiving even more nicotine and level of carcinogens compared to when he was smoking.

Your third and final mission is to uncover why Corey would smoke more reduced-nicotine cigarettes compared to regulars, and why he would receive more harmful chemicals if he used the smokeless tobacco. Consult the Module 3 guide to answer the questions below for Mission #3.

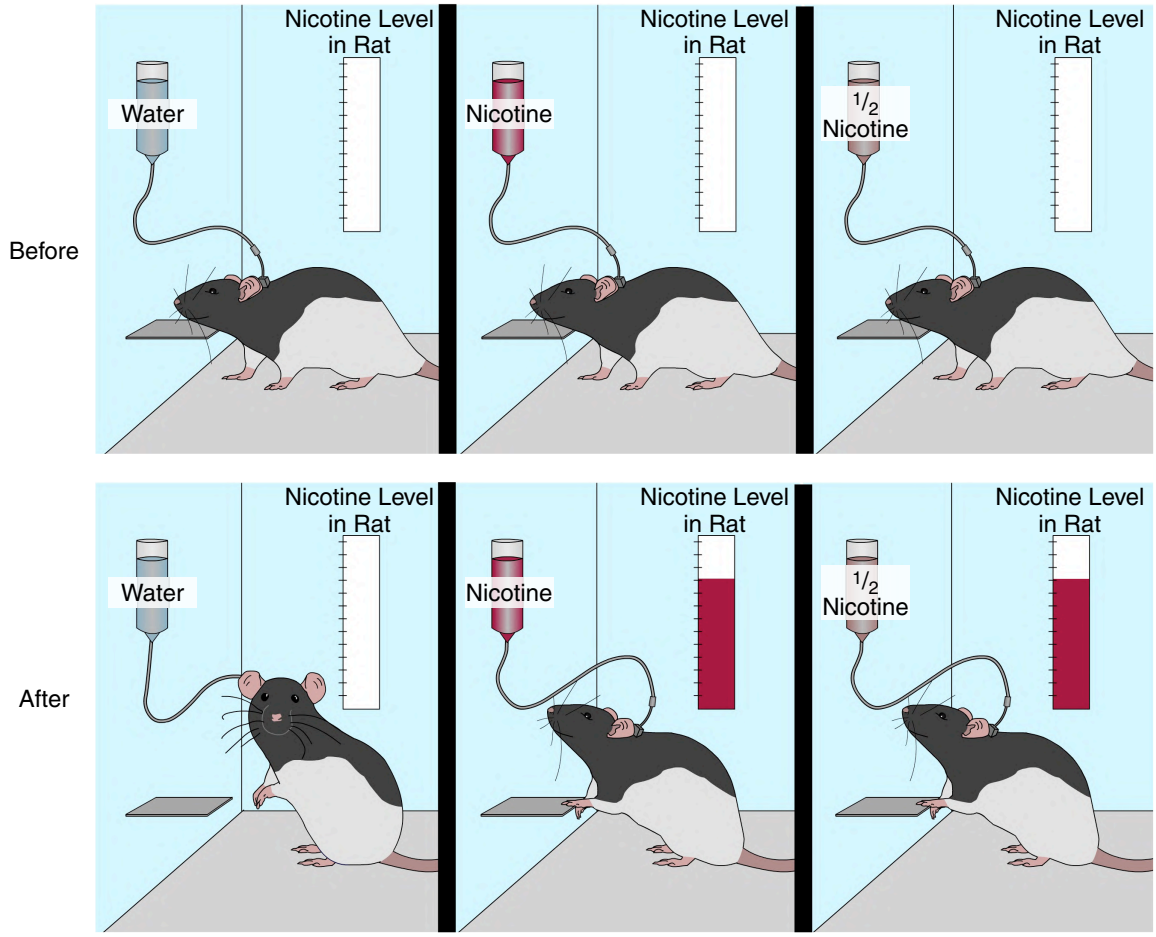
Try the experiment in question #1 to guide you in your quest for knowledge.

Name _____ Group #_____

Mission #3

1. Animals such as rats enjoy nicotine just like humans. In experiments, rats will press a lever in a box to get an injection of nicotine. The pictures below shows a typical experiment. The 3 rats can press a lever to receive an injection of either A) water, B) water containing nicotine, or C) water containing half as much nicotine. After the experiment, in the lower panel, it shows how much nicotine each rat received.

   If you are viewing this page on a computer, **click on the top picture** to see how often each rat presses the lever, and how much nicotine each rat receives. You may have to watch the animation closely a few times to see what's happening.



   How much nicotine does the rat on the right get when it presses the lever for the bottle containing half the amount of nicotine? Circle your answer.

   a. half as much as the rat gets in the middle panel

   b. twice as much as the rat gets in the middle panel

   c. the same as the rat gets in the middle panel

How fast did the rat on the right press the lever to get nicotine?

a. half as fast as the rat pressed in the middle panel

b. twice as fast as the rat pressed in the middle panel

c. the same rate as the rat pressed in the middle panel

2. When Corey tried to cut down his smoking early on, he bought some "reduced-nicotine cigarettes". He thought this would help him quit.

   Do you think that he spent more or less money on cigarettes each week? _____

3. From the list of chemicals below, write in which ones are found in each of the products shown in the table:

Benzo[a]pyrene	**Nicotine**	**Nitrosamines**	**Carbon Monoxide (CO)**	**Tar**

   Hint: Be sure to consult your manual to determine how each of the products are formed (i.e., produced only when smoked, etc.) You may need to refer to Chapter 2 to answer parts of this question.

Smokeless Tobacco	Reduced-Nicotine/Tar Cigarette	Snus	Regular Cigarette	Nicotine Patch	Tobacco Lozenge

4. There is one ingredient in all the products listed above that is common to each. What is it?_____.

   Explain why this ingredient must be present in any product to ensure that someone would want to use that product.

   Give 2 reasons why this ingredient is harmful:

   1.

   2.

5. By now you have probably figured out why Corey received more carcinogens when he switched from smoking to smokeless tobacco. What could explain this?

The cigarette is probably the most widely used nicotine delivery device on the market. However, the tobacco industry has released a host of other kinds of tobacco-containing products, as well as non-tobacco nicotine-delivery products. How do these products compare to cigarettes? Do people who use these products also get addicted? A brief review of some of these products can shed some light on these questions.

The following topics will be discussed in this section:

- Reduced-nicotine/tar cigarettes: a healthier alternative to regular cigarettes?
- Smokeless tobacco: can it cause addiction and cancer?
- Nicotine-delivery products: are they safer?

Reduced-nicotine/tar cigarettes: a healthier alternative to regular cigarettes?

Tobacco companies have created cigarettes that are supposed to deliver a lower amount of **nicotine** and **tar** (**carcinogens**) compared to regular cigarettes. Although the labels on packs of these cigarettes show how much nicotine is in each cigarette, the labels are misleading.

nicotine
The highly addictive chemical found in tobacco products.

tar
The mixture of toxic chemicals found in the cigarette smoke.

carcinogen
Any chemical that is known to cause cancer in animals and humans.

The nicotine and tar contents are determined with "smoking machines", which do not reflect how people smoke. For example, reduced-nicotine/tar cigarettes were designed to have tiny air holes in the filter section that help dilute the smoke and nicotine. The idea is that, in theory, users will inhale more air and less nicotine and tar.

However, the air holes are positioned strategically so that when a smoker actually holds the cigarette, his/her fingers naturally cover the tiny air holes. Thus, the smoker inhales more of the nicotine and tar and less air. The smoking machine does not block these air holes, so the nicotine and tar content measured by the machine are actually lower than what an actual smoker receives.

When people smoke cigarettes, or use any tobacco product, they use enough of the tobacco product to obtain a certain level of nicotine in their brains that makes them feel good. As discussed previously, nicotine binds to nicotinic receptors, activating the neurons to fire electrical signals in the part of the brain responsible for pleasurable feelings. So, to get the expected effects of nicotine, there must be enough of the drug in the brain to bind to the receptors.

What do you think will happen if the cigarette contains less nicotine? Take a look at **Figure 3.1**. Smokers of the reduced-nicotine cigarettes

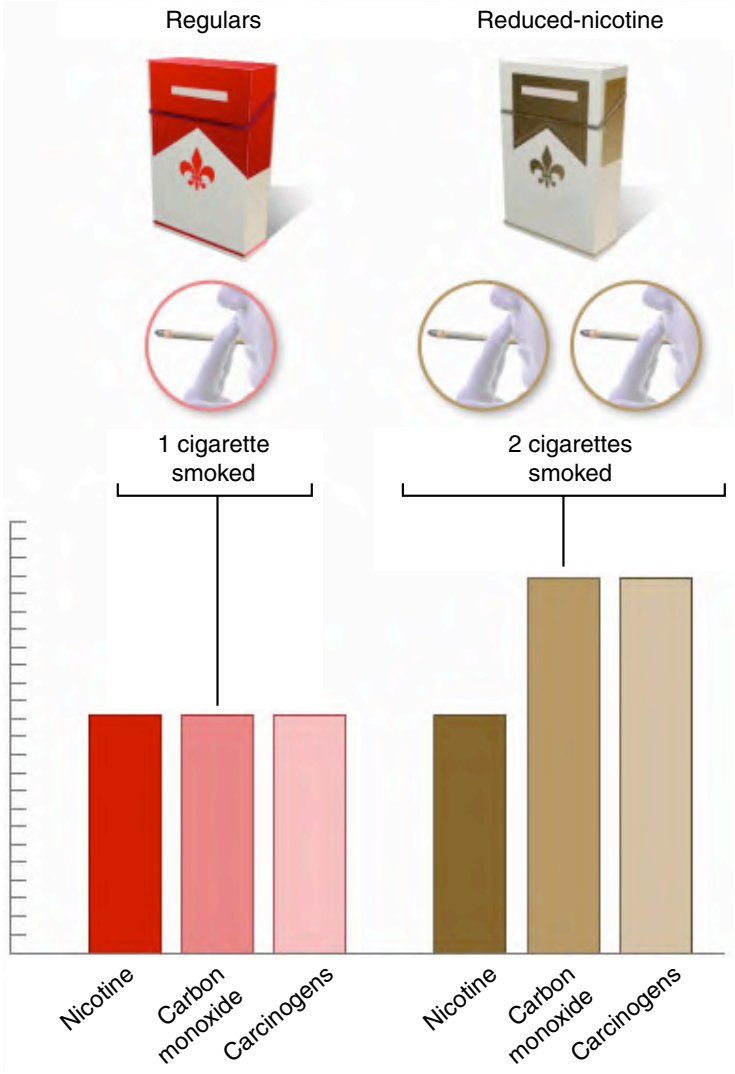


Figure 3.1 Smokers smoke cigarettes to attain an effective nicotine level. The reduced-nicotine cigarettes on the right have less nicotine compared to the regular cigarettes on the left. People tend to smoke more of the reduced-nicotine cigarettes to get the same level of nicotine in their brain. In doing so, they inhale more carbon monoxide and carcinogens.

automatically compensate by inhaling more often and taking longer and deeper puffs to get the needed nicotine. In the end, the smoker may actually smoke more of the reduced nicotine/tar cigarettes in order to receive the amount of nicotine they need.

These reduced-nicotine cigarettes are also labeled with less tar, or carcinogens. But because the smoker needs to inhale more deeply, take more puffs, or smoke more of these cigarettes to get the needed amount of nicotine, they also end up inhaling more carcinogens—even if the cigarettes do have less tar. Not only do they inhale more carcinogens, they also inhale more carbon monoxide too.

As discussed above, it only takes one carcinogen and one mutation to cause cancer, regardless of how many carcinogens one might inhale. In fact, scientific studies have found that there is actually no statistical

difference in health risk of smokers of reduced-nicotine/tar cigarettes versus regular cigarettes.

Smokers of reduced-nicotine cigarettes will compensate by smoking more to get the level of nicotine that is typically delivered by regular cigarettes. They get more carbon monoxide and carcinogens too.

Smokeless tobacco: can it cause addiction and cancer?

There are a number of other tobacco products on the market besides cigarettes. Different products appeal to different people for a variety of reasons. Such products include **smokeless tobaccos** (including chewing tobacco, **snuff**, **snus**, and lozenges), cigars, little cigars, pipes, and water pipes.

smokeless tobacco
Tobacco, either shredded or ground that can be placed in the nose or mouth. Common forms include chewing tobacco, snus, and snuff. Slang names include rug, plug, chew, spit, and dip.

snuff
Finely ground tobacco leaves that can be either dry, moist, or come in small pouches. Also known as dipping or spit tobacco.

snus
A moist powdered tobacco that is packaged into small pouches, similar to tea bags and can be placed between the lip and gums to release nicotine without generating saliva juices.

Each of these products containing tobacco is harmful because they all contain nicotine and carcinogens. Let's look at a few examples of smokeless tobacco.

Tobacco Products

- *Regular or menthol cigarettes*
- *Reduced nicotine/tar cigarettes*
- *Cigars, little cigars*
- *Pipes*
- *Chewing tobacco*
- *Snus tobacco*
- *Water pipe*
- *Tobacco lozenges*

Chewing tobacco

One of the main types of smokeless tobacco in the United States is chewing tobacco. Chewing tobacco consists of whole or shredded tobacco leaves that are placed between the cheek and gum of the user.

Nicotine and other chemicals are released from the tobacco leaves into the saliva when the user chews on the tobacco (hence the term "chewing tobacco"). The nicotine and other chemicals in tobacco are absorbed through the cheek cells into the bloodstream. In addition, the chewing of the tobacco produces excess juice in the mouth, which the user must either spit out or swallow.

A common misconception about smokeless tobacco is that it is not as harmful as smoking because it contains less harmful chemicals. This is not the case for smokeless tobacco, otherwise known as "chew" or "dip".

Smokeless tobacco contains at least 30 known cancer-causing chemicals. The most harmful of these chemicals are the **nitrosamines**, which are made during the fermenting and aging process of tobacco (some nitrosamines can be generated from the breakdown of nicotine).

nitrosamines
Chemical compounds found in tobacco products that are highly carcinogenic; two well known examples include NNK and NNN.

Research shows that people who use smokeless tobacco may actually be exposed to higher levels of the nitrosamines, depending on the brand and length of time held in the mouth. One of the main reasons for the higher amount of toxins in the body is that the tobacco stays in the mouth longer than one smokes a cigarette, so the harmful chemicals have more time to be released into the body.

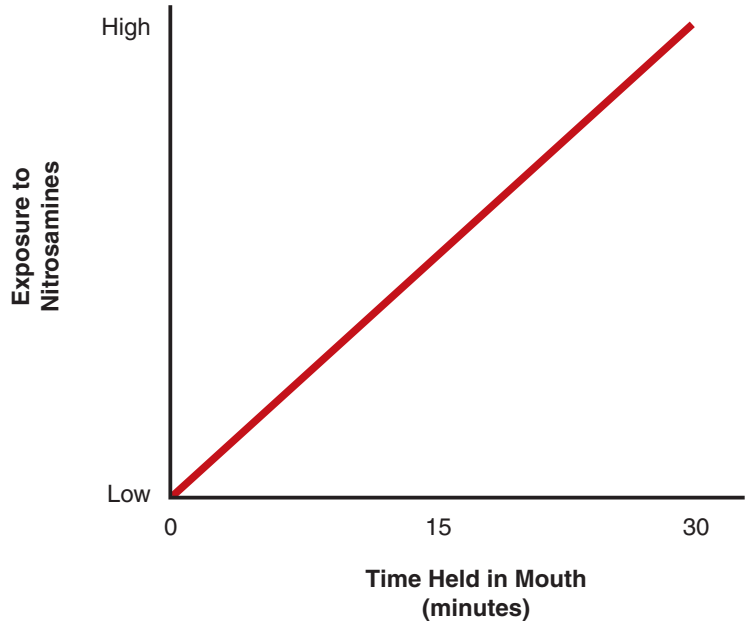


Figure 3.2 Carcinogen exposure from smokeless tobacco. Users of smokeless tobacco can receive more nitrosamine carcinogens and nicotine compared to smokers.

Although smokeless tobacco contains fewer carcinogens compared to cigarettes, all it takes is one carcinogen to cause a mutation that leads to cancer. Contrary to popular belief, smokeless tobacco can actually lead to many types of cancers, including oral, pancreatic, esophageal, and even lung cancer.

Long-term use of smokeless tobacco can also cause bleeding of the gums and mouth, separation of the gums from the teeth (which over

time could lead to teeth decay), yellowing of the teeth, bad breath, increased heart rate, and high blood pressure.

Users of smokeless tobacco also receive more nicotine compared to smoking a cigarette. Because smokeless tobacco users tend to leave the tobacco in their mouths for about 30 minutes, the amount of nicotine absorbed into the body from a typical tobacco chew or "dip" is approximately 3-4 times the amount absorbed from smoking a single cigarette.

If the user is exposed to higher amounts of nicotine compared to cigarettes, then tolerance and dependence to nicotine will occur faster. The user will not be able to stop using the tobacco product without going through some withdrawal reactions. The ability to quit using tobacco becomes quite difficult. Moreover, many smokeless tobacco users are at high risk of becoming cigarette smokers.

Teenage users of smokeless tobacco have a higher risk of becoming cigarette smokers within 4 years than non-users.

Snus

Another type of smokeless tobacco is called *snus* (rhymes with goose), which is the Swedish word for snuff. Snus tobacco is a moist powdered tobacco that is packaged into small pouches, similar to tea bags. These pouches are placed between the user's lip and gums. The nicotine and other compounds in the tobacco leach out of the packets, and move through the gums into the bloodstream. Snus does not produce extra juices in the mouth, so snus users don't feel the need to spit.

Snus has been sold in Sweden for many years, but it is banned for sale in all other European countries. Recently, many American tobacco

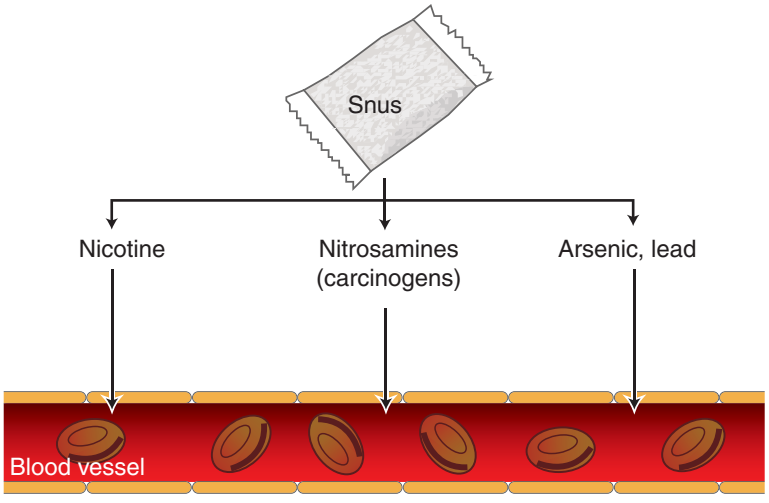


Figure 3.3 Chemicals in snus are absorbed into the bloodstream. Snus, small packets of tobacco held against the gums, release nicotine, cancer-causing nitrosamines, and other toxic compounds into the bloodstream.

companies have designed their own version of the snus and have released this new product into the US market. However, the US snus is quite different from the Swedish form. It contains up to 60 times the levels of cancer-causing nitrosamines (depending on the brand) compared to the Swedish forms.

Some claim that people who use snus don't get cancer. However, there are no long term studies to actually prove this—large numbers of people have not been using snus for long enough (like 30 years) to detect the cancer.

Nevertheless, recently, even the Swedish form, with lower levels of nitrosamines, has been shown to cause pancreatic cancer.

Another difference from the Swedish snus is that the American snus contains lower amounts of nicotine. As discussed above with reduced-nicotine cigarettes, the lower the nicotine, the more of the product the user needs to maintain the desired amount of nicotine in her/his brain.

US brands of snus have higher nitrosamines and lower nicotine compared to Swedish snus.

Tobacco Lozenges

Tobacco lozenges are compressed powered tobacco products that are designed to dissolve in the mouth, similar to a lozenge or hard candy. These lozenges have minty flavorings and are about the size of a Tic-Tac™.

Given their size, people may think that the lozenges are not harmful. On the contrary, lozenges are made from the same tobacco found in chewing tobacco and cigarettes, and contain the same carcinogens, such as nitrosamines. As the lozenge is held in the mouth, the nitrosamines and nicotine are absorbed through the cells lining the mouth, into the bloodstream.

Just like with other tobacco products, repeated use can lead to nicotine addiction. Again, since this product is relatively new on the market, it will take a few decades to uncover whether cancer emerges, especially of the mouth.

Addiction and Cancer

All forms of tobacco, whether in a cigarette, pipe, dip, snus, or lozenge, contain the same harmful ingredients. These include nicotine, nitrosamines, arsenic, lead, and other metals.

Makers of different products claim that there are reduced forms of carcinogens such as nitrosamines compared to cigarettes, leading some smokers to believe that switching to a smokeless product may be a

better alternative to smoking. But cancer is not a disease that develops based on the amount of the carcinogen; ANY exposure can cause mutations that lead to tumor formation and cancer. So even the presence of low levels of a single carcinogen can increase one's risk of getting cancer, especially with long-term use.

Additionally, the presence of the nicotine in each of these products provides pleasure to the user, and the user will develop a pattern of use to deliver the amount of nicotine necessary to feel good. So the risk of developing dependence and addiction to the product still exists, in addition to the continued exposure to the toxic chemicals.

Nicotine-delivery products: are they safer?

In addition to smokeless tobacco products, there are several types of nicotine-delivery products on the market. For example, the nicotine patch, gum, and inhaler have been approved by the **Food and Drug Administration** for use as "nicotine-replacement therapy" by people trying to quit smoking. On average, using a form of nicotine-replacement therapy can double one's chance of giving up tobacco for good. Yet the percentage of tobacco users who actually quit is low, so that leaves millions of users still addicted to nicotine.

Food and Drug Administration
US government agency that regulates the safety and security of our nation's food and drug supply.

Science studies have shown that nicotine-replacement products appear to be a safer alternative to using smokeless tobacco products or modified cigarettes for helping people quit smoking because they don't contain tobacco.

However, the nicotine-replacement product must be used in a specific way to avoid withdrawal—the user weans off the product over several weeks or months by slowly lowering the dose of nicotine that enters the body. The products are not intended for long-term use.

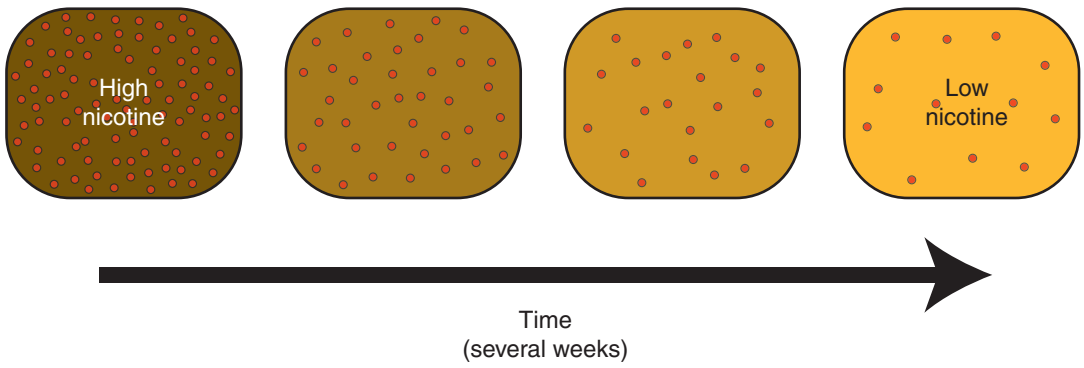


Figure 3.4 The nicotine patch: a quitting device. The nicotine patch helps users quit when they slowly wean off the nicotine.

Scientists do not yet know whether a person using nicotine (by itself) for many years will develop cancer or heart disease, because people have

not been using nicotine-only products for long enough (for example, 30 years) to do the studies. A surprising study released in 2009 indicates that long term use of the nicotine patch can actually cause low levels of nitrosamine carcinogens to be formed from the breakdown of nicotine inside the body.

Thus, we will have to wait at least another decade to know whether using nicotine, which has been shown to cause DNA mutations, prevent the death of cells with damaged DNA, and promote tumor growth in mice, will do the same in humans.

Lab

Can Tobacco Cause Mutations in DNA?

Can Tobacco Cause Mutations in DNA?
A Virtual Experiment

Target Group: Health Education or Biology for Grades 9–10

SUMMARY

Objectives

The major objective in this experiment is to determine if compounds in tobacco can cause DNA mutations in a strain of bacteria (*Serratia marcescens*), in as little as 2 days. Mutations in bacterial DNA cause the bacteria to change from red to white. After completing the exercise, the student should be able to:

- Discuss the effect of tobacco on DNA
- Describe how a DNA mutation is manifested in *S. marcescens*
- Explain how fast a DNA mutation can occur
- Explain how one could determine if nicotine can cause mutations in the same system

HYPOTHESIS AND EXPERIMENTAL DESIGN

To address the objective of this exercise the following hypothesis can guide the experiment:

> *Compounds in tobacco will cause mutations in the DNA of the bacteria,* Serratia marcescens*, within 2 days.*

To test the hypothesis, one needs to design how to set up the experiment, including conditions that will serve as "controls". In this experiment, there are be 3 experimental conditions to measure DNA mutations in *S. marcescens*: 1) extract of tobacco (in water), 2) UV light exposure, and 3) water alone. The water controls for possible mutations produced by something in the water that is used to make the extract. Second, the UV light causes mutations in many organisms, including humans. It is used to show that the experiment actually works…the UV light should cause DNA mutations regardless of what happens with the tobacco condition. OK, now let's take a look at the actual procedure.

MATERIALS

- Online access to the 10 minute "virtual lab", performed by peer students (www.rise.duke.edu/seek).
- Color printouts of the 3 agar plates containing bacterial colonies.

METHODS

Serratia Marcescens is a strain of bacteria commonly found in the environment. *S. Marcescens* produces a red pigment (i.e. colonies are red) when grown at 24-30° C. Mutations in the bacterial DNA for the red pigment will cause the colonies to turn white (they don't make the red pigment) or pink (they make less of the red pigment).

UV light is used as a control condition to show that the experiment works. UV radiation causes mutations in the bacterial DNA and the colonies should turn white. (UV light will also kill some of the colonies).

Watch the video

What's not in the video: The preparation

Prior to the actual experiment shown in the video, the teacher carried out some preparation. The preparation included the following:

- Make stock plates of bacteria by inoculating agar (nutrients in gelatin) petri plates with the bacteria purchased from a biological company
- Store the plates for 2 days at room temperature (24-25°C)
- Transfer a single colony of bacteria to a tube containing liquid nutrients (nutrient broth)
- Store the liquid bacteria culture for 1 day before starting the experiment
- Before starting the experiment, dilute the bacteria (1 drop) in a new tube containing 10 milliliters of fresh nutrient broth. Then repeat. This dilutes the bacteria to be used for the experiment.

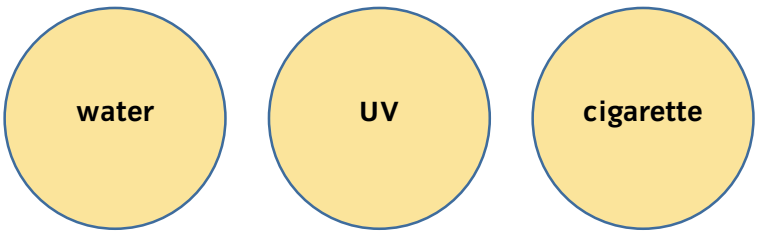
What's in the video: Experiment day

Step 1: Students make the tobacco extracts

- Heat water, cut open a cigarette and put tobacco in the water, stirring for 5 minutes
- Heat another beaker of water for the control
- Filter the extract through cheesecloth into a bottle

Step 2: Students add tobacco extracts to plates

- Label the underside of 3 agar petri plates as follows:



- Place 1 drop of the following solutions in the middle of each agar plate

  Water plate: add previously heated water

  UV plate: add previously heated water

  Cigarette plate: add cigarette extract (in water)

You might ask why water is added to the UV plate. The reason is that with this design all 3 plates contain the same water, providing a fair comparison.

- Use glass beads to roll the solution all over the plate, let solution dry for ~20 minutes

Step 3: Add bacteria to the plates

- Place 1 drop of bacterial solution into the middle of each plate
- Spread the bacteria on the plate using fresh glass beads; let dry for ~ 10 minutes
- For the UV plate, place the UV lamp over a box with a hole in the top and shine the UV light through the hole onto the plate for 15–20 seconds. This should cause mutations in the bacterial DNA. It will also kill many bacterial cells.
- Store the bacterial plates for 2 days at room temperature
- Take pictures of the plates and send to a computer

Collect Data: Done in class, this is no longer virtual

Data collection is done 2 days after the bacteria were treated; You will count bacterial colonies in each plate that was prepared by the students in the video to determine the number of mutations.

- Indicate on your lab datasheet (included at the end) what you expect to find BEFORE looking at the plates.
- Your teacher will hand out pictures of Plates A, B, and C, to each group; these are the actual results from the experiment that the students did in the video.
- The plates are coded A, B, and C, so that no bias will be introduced when counting colonies. You should not know which plate is which until after the data are collected.
- Count the number of white spots on the printout of each plate. An example is found on the last page.

  red = normal DNA white = mutated DNA

- Record your numbers in the data table that is found in your lab datasheet.
- After you have finished counting, your teacher will "break the code" and tell you which plate is which. Write the actual treatment in the table under the code letters.
- Answer the questions on your datasheets for a class discussion about the experiment.

STUDENT DATA SHEET Name: _____

Before you start, what do you expect to see? Circle the level of mutations you expect for each plate:

Water plate	No mutations	A few mutations	A lot of mutations
UV-exposed plate	No mutations	A few mutations	A lot of mutations
Cigarette extract plate	No mutations	A few mutations	A lot of mutations

---

Now count the number of white spots on the entire plate.

Record your data in the following table:

Plate Treatment	# of white colonies on plate
Plate A	
Plate B	
Plate C	

Remember: red = normal DNA white = mutated DNA

Based on your results, what do you think was added to each plate (water, UV light, or cigarette extract)?

Plate A: _____Plate B: _____Plate C: _____

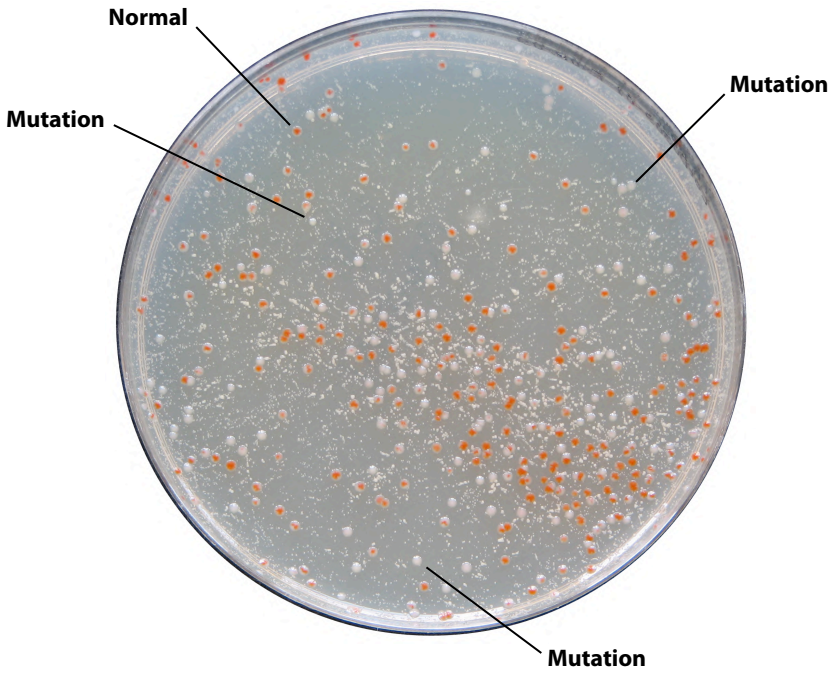
Explain in 2-3 sentences why you chose your answers.

Now, ask your teacher to identify the conditions for each plate. Write them into the table in the first column. Did you guess correctly which plate was which? If not, what are some reasons why your answers were wrong?

Go back and look at your predictions (before you counted colonies) for the level of DNA mutations produced in each condition. Which of your predictions were not supported by the results of the experiment?

What are some reasons why the experimental results did not support your predictions?

Sample Agar Plate Image for Counting



Glossary

Acetylcholine A major neurotransmitter in the brain that provides signals for a variety of actions, including level of alertness, learning, and memory. Also located in neurons outside the brain, it provides signals to the heart, blood vessels, and other muscles to contract.

Addiction The compulsive use of a drug, despite the experience of negative consequences—no control over use.

Alveoli Tiny air sacs in the lung that participate in the gas exchange of carbon dioxide and oxygen.

Apoptosis A form of cell death that is triggered by a series of signals originating in the cell's own genetic instructions ("cell suicide"). Apoptosis is the cell's mechanism to destroy itself when there is an error or mutation in its DNA.

Artery Blood vessel that carries blood from the heart to all organs in the body.

Axon A long extension from the cell body of a neuron that carries electrical signals toward the terminal .

Benzo[a]pyrene (BaP) A carcinogen found in tobacco smoke that is produced by the burning of cigarettes; it is found in many combustion reactions such as the burning of fuel.

Brain The organ enclosed in the skull that carries out all cognitive and mental functions.

Cancer Abnormal and uncontrolled growth of cells in the body.

Capillaries The smallest type of blood vessel found in the body.

Carbon dioxide Chemical gas composed of one carbon molecule and 2 oxygen atoms (CO_2) that is produced by all cells in the body as a waste product; it is released in the breath by exhaling.

Carcinogen Any chemical that is known to cause cancer in animals and humans.

Cell body The main part of the neuron (major cells in the brain) that contains the nucleus, in which the genetic material is found.

Chewing tobacco A form of smokeless tobacco formed from shredded or whole tobacco leaves that is placed in the mouth and chewed to release the nicotine and flavors from the tobacco.

Chromosome Compact structure of DNA and proteins located in the nucleus of a cell. Every cell in the human body contains 23 pairs of chromosomes.

Compulsive An intense urge to perform an action, with little control to stop the action.

DNA A large molecule of heredity material that contains the genetic information; DNA, or deoxyribonucleic acid, is found in the nucleus of every cell.

Dendrite Short branch-like extensions of the cell body that receive chemical signals from other nearby neurons .

Dependence The user (or organism) functions normally only in the presence of the drug; removal of the drug results in negative symptoms (withdrawal). Dependence develops with repeated use of the drug.

Dopamine A neurotransmitter that is involved in the brain's response to emotions, movement, and ability to respond to feelings of pleasure. The pleasurable response to addictive drugs results from stimulation of dopamine action within the brain's reward pathway.

Emphysema A lung disease with symptoms that include shortness of breath and difficulty breathing. Emphysema is due to damage to the air sacs in the lungs and in the airway, often caused by the buildup of toxic chemicals found in cigarette smoke.

Food and Drug Administration US government agency that regulates the safety and security of our nation's food and drug supply.

Genes Segments of DNA that carry the complete genetic information (instructions) to make a protein.

Heart The organ responsible for pumping blood throughout the body.

Little cigar A tightly rolled bundle of tobacco with an outer wrapping of tobacco leaves (also called small cigars).

Lung The organ in the body responsible for carbon dioxide and oxygen exchange.

Lung cancer Uncontrolled growth of cells in the lung. More than 80% of lung cancer cases are due to the use of tobacco.

Metastasis Movement of cancer cells away from the primary tumor site to different locations within the body.

Mucous membranes Line the inside of passages into the body (e.g., nose, stomach); made up of a type of skin cells that can secrete mucous, which protect the cells lining the tissue.

Mutation Change in the DNA sequence caused by environmental or genetic factors .

National Cancer Institute US government agency specializing in cancer research, treatment, and other cancer-related policies .

Neuron A nerve cell that communicates with other cells (often neurons) using electrical and chemical signals. A neuron contains a cell body, an axon, and many dendrites.

Neurotransmitter A chemical released from one neuron and binds to receptors on another ("chemical messenger"). Two major neurotransmitters include acetylcholine and dopamine.

Nicotine The highly addictive chemical found in tobacco products.

Nicotinic receptor A protein that binds both acetylcholine and nicotine to increase the electrical activity of neurons.

Nitrosamines Chemical compounds found in tobacco products that are highly carcinogenic; two well known examples include NNK and NNN.

NNK A carcinogen found specifically in tobacco that belongs to a family of chemicals called nitrosamines. NNK is produced during the curing (or preservation) process of tobacco used in cigarettes, smokeless tobacco, and other products.

Nucleotides Building blocks of DNA. DNA is composed of strings of 4 nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C).

PET scan Technique used to visualize and measure blood flow, cellular activity, or receptor density in the brain.

Prefrontal cortex The frontal region of the brain involved in thinking, impulsive and compulsive behavior, and decision making. It is one of the last areas of the brain to develop.

p53 (protein) A protein that provides signals to the cell to undergo apoptosis ("suicide") when it has mutations in its DNA. It is one of the major proteins that suppresses the formation of tumors.

Protein A functional molecule formed by specific instructions contained within the DNA. There are approximately 30,000 different proteins made from DNA; each protein has a unique function in the body.

Receptor A protein to which hormones, neurotransmitters, and drugs bind. Receptors are usually located on cell membranes and elicit a function once bound.

Second-hand smoke Refers to the tobacco smoke inhaled by someone in close proximity to the smoker without actually smoking the tobacco product (cigarette, pipe, cigar, etc.)

Smokeless tobacco Tobacco, either shredded or ground that can be placed in the nose or mouth. Common forms include chewing tobacco, snus, and snuff. Slang names include rug, plug, chew, spit, and dip.

Snuff tobacco Finely ground tobacco leaves that can be either dry, moist, or come in small pouches. Also known as dipping or spit tobacco.

Snus A moist powdered tobacco that is packaged into small pouches, similar to tea bags and can be placed between the lip and gums to release nicotine without generating saliva juices.

Stroke The brain becomes deprived of oxygen and glucose, either because of a blockage in a brain blood vessel, or a ruptured blood vessel. A person with a stroke can lose some or all sensation and movement. High blood pressure and heart disease can lead to a stroke.

Synapse The connection between the two neurons; it is where all the communication between neurons happens.

Tar The mixture of toxic chemicals found in the cigarette smoke.

Tobacco lozenges Mint flavored lozenge containing compressed tobacco powder.

Tolerance Results when a person (or organism) requires a higher dose to get the same effect; or, the same dose no longer produces the same level of effect that was present initially.

Telomeres Small strands of DNA at the ends of the chromosomes that protect the DNA from damage during cell division. The telomeres shorten with each cell division, and if they become too short, the DNA is no longer protected.

Tumor Uncontrolled growth of cells that leads to the formation of a large mass of tissue.

Withdrawal Negative side effects felt by a habitual drug or tobacco user when the substance is no longer present in the body. Nicotine withdrawal symptoms include irritability, hand tremors, anxiousness, inability to sleep, and depressed mood.

World Health Organization A world-wide organization that is the leading authority in making health decisions that affect countries throughout the world.

Resources and References

Anatomy of a cigarette as designed by Dr. Wigand, a tobacco industry insider

http://jeffreywigand.com/cigarette.php

Animated and clickable image of a cigarette.

Campaign for Tobacco-Free Kids

http://www.tobaccofreekids.org

Excellent resource for information relating to tobacco use, adolescents and smoking prevention. Also contains press releases for many news stories regarding the tobacco industry and marketing to teens.

Cell Biology and Cancer. National Institute of Health Curriculum Supplement Series

http://science.education.nih.gov/Customers.nsf/HSCancer?OpenForm

Curriculum supplement for use in the classroom. Includes various classroom activities related to cancer. Appropriate for grades 9–12.

Mayo Clinic animation of how smoking causes lung cancer

http://www.msnbc.msn.com/id/11030549/

National Cancer Institute

http://www.cancer.gov/cancertopics/smoking

Website with various facts, statistics and latest research relating to cancers related to tobacco products.

National Geographic animation of lung function and carbon dioxide and oxygen air exchange

http://science.nationalgeographic.com/science/health-and-human-body/human-body/lungs-article.html

Animations describing the detailed function of the lungs and air exchange. Includes interactive features as well as excellent graphics of lung structure and function.

National Institute of Drug Abuse for Teens

http://teens.drugabuse.gov/index.asp

Contains great activities and information for both students and teachers in all grade levels.

National Institute of Drug Abuse: The Brain: Understanding Neurobiology through the Study of Addiction

http://www.nida.nih.gov/Curriculum/HSCurriculum.html

Excellent science background and science-based activities for students in grades 9–12.

National Institute of Drug Abuse: Mind over Matter: Nicotine

http://teens.drugabuse.gov/mom/mom_nic1.asp

Science based curriculum developed for grades 5–9.

National Institute of Drug Abuse: The Science of Drug Abuse and Addiction

http://www.nida.nih.gov/

National Institute of Health: Office of Science Education: Understanding Cancer:

http://science.education.nih.gov/home2.nsf/SearchDocs?SearchView&Query=cancer&

An excellent review on cancer in the form of a PowerPoint™ presentation.

Search for a Safer Cigarette

http://www.pbs.org/wgbh/nova/cigarette/

PBS NOVA program on tobacco industry's attempt to create a "safer" cigarette.

Smoking in adolescent fact sheet

http://www.lungusa.org/site/c.dvLUK9O0E/b.39871/

Surgeon general flash animation of smoking damages every organ of the body

http://www.cdc.gov/tobacco/data_statistics/sgr/sgr_2004/sgranimation/html/index.html

Compare healthy and diseased organs. Contains a lot of information and facts concerning diseases related to tobacco. Interactive. Requires Adobe Flash player.

2004 Surgeon General's Report: The Health Consequences of Smoking

http://www.cdc.gov/tobacco/data_statistics/sgr/sgr_2004/00_pdfs/SGR2004_Whatitmeanstoyou.pdf

Excellent resource for information regarding the health effects of smoking as well as a resource for quitting smoking and tobacco products.

Scientific References

Module 1

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National Health and Science Education Standards

National Health Education Standards

The National Health Education Standards are found at the Center for Disease Control and Prevention: http://www.cdc.gov/healthyyouth/sher/standards/index.htm

The following health education standards apply to SEEK:

Standard 1

Students will comprehend concepts related to health promotion and disease prevention to enhance health.

- Predict how healthy behaviors can affect health status.
- Describe the interrelationships of emotional, intellectual, physical, and social health.
- Analyze how environment and personal health are interrelated.
- Analyze how genetics and family history can impact personal health.
- Propose ways to reduce or prevent injuries and health problems.
- Analyze personal susceptibility to injury, illness, or death if engaging in unhealthy behaviors.
- Analyze the potential severity of injury or illness if engaging in unhealthy behaviors.

Standard 2

Students will analyze the influence of family, peers, culture, media, technology, and other factors on health behaviors.

- Analyze how peers influence healthy and unhealthy behaviors.
- Evaluate the effect of media on personal and family health.
- Analyze how some health risk behaviors can influence the likelihood of engaging in unhealthy behaviors.

Standard 3

Students will demonstrate the ability to access valid information, products, and services to enhance health.

- Evaluate the validity of health information, products, and services.

Standard 5

Students will demonstrate the ability to use decision-making skills to enhance health.

- Predict the potential short-term and long-term impact of each alternative on self and others.
- Defend the healthy choice when making decisions.
- Evaluate the effectiveness of health-related decisions.

Standard 7

Students will demonstrate the ability to practice health-enhancing behaviors and avoid or reduce health risks.

- Demonstrate a variety of healthy practices and behaviors that will maintain or improve the health of self and others.
- Demonstrate a variety of behaviors to avoid or reduce health risks to self and others.

National Science Education Standards

The complete National Science Education Standards can be found at:
http://www.nap.edu/openbook/0309053269/html/111.html

SCIENCE CONTENT STANDARDS (C)

Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry
- Design and conduct scientific investigation

Life Science

- The cell
- Molecular basis of heredity
- Behavior of organisms

Science in Personal and Social Perspectives

- Personal and community health

ASSESSMENT STANDARDS (A)

***ASSESSMENT STANDARD A**: Assessments must be consistent with the decisions they are designed to inform.*

- Assessments are deliberately designed.

ASSESSMENT STANDARD B: Achievement and opportunity to learn science must be assessed.

ACHIEVEMENT DATA COLLECTED FOCUS ON THE SCIENCE CONTENT THAT IS MOST IMPORTANT FOR STUDENTS TO LEARN. The content standards define the science all students will come to understand. They portray the outcomes of science education as rich and varied, encompassing

- The ability to inquire.
- Knowing and understanding scientific facts, concepts, principles, laws, and theories.
- The ability to reason scientifically.

- The ability to use science to make personal decisions and to take positions on societal issues.

- The ability to communicate effectively about science.

ASSESSMENT STANDARD C: The technical quality of the data collected is well matched to the decisions and actions taken on the basis of their interpretation.

- Students have adequate opportunity to demonstrate their achievements.

SCIENCE TEACHING STANDARDS (T)

TEACHING STANDARD B: Teachers of science guide and facilitate learning. In doing this, teachers

- Focus and support inquiries while interacting with students.

- Orchestrate discourse among students about scientific ideas.

TEACHING STANDARD C: Teachers of science engage in ongoing assessment of their teaching and of student learning. In doing this, teachers

- Guide students in self-assessment.

TEACHING STANDARD D: Teachers of science design and manage learning environments that provide students with the time, space, and resources needed for learning science. In doing this, teachers

- Create a setting for student work that is flexible and supportive of science inquiry.

- Make the available science tools, materials, media, and technological resources accessible to students.

- Engage students in designing the learning environment.

TEACHING STANDARD E: Teachers of science develop communities of science learners that reflect the intellectual rigor of scientific inquiry and the attitudes and social values conducive to science learning. In doing this, teachers

- Nurture collaboration among students.

For Teachers

Teacher's Instructions

Materials:

SEEK about Tobacco Unit (either as the website or the pdf printed out)

Handouts (the "Stories", "the Missions", and the Lab)

Bacterial plate pictures (for collecting data)

Computer(s) (optional, but preferred)

Internet access to the video of the "Virtual Lab"

LCD Projector (optional)

Preparation:

Have your classroom computer, or laptop cart available for the unit. Ideally, students should work in pairs, so 1 laptop per pair would be best. Make copies of the handouts (Stories, Missions, and Lab) and the color bacterial plate pictures, if needed.

Implementation

The unit can be completed in 2-3 class periods, or performed as an out-of-class group homework activity.

Start with Module 1 – have students read the "Story" (to themselves) and then answer the series of questions in the "Mission." Students can use the SEEK about Tobacco unit (or they can access it online at http://www.rise.duke.edu/seek) to find the answers. The answers to the mission questions are found in the "Downloads for Teachers" PDF.

Ideally, it is best to have a computer with projector, or a computer available for students to use, especially since some figures are animated. If no computer is available, any animated figures will still show the key images with the same content as in the animation.

Notes about the lab activity (Can Tobacco Cause Mutations in DNA ?)

Module 2 includes a laboratory experiment. The lab exercise is performed "virtually." Have students watch the 10 minute video on our website at http://www.rise.duke.edu/seek showing a group of peers actually performing the lab (this is where the LCD projector comes in handy.) Your students can collect and analyze the data generated by the students in the video. The bacterial plates have been photographed and are included in the lab section--students can count the colonies on those pictures.

Specific instructions for the lab experiment are found in the "Downloads for Teachers" PDF. After completing the lab datasheets, students may hand in the lab results (including answering the questions on the data sheet) to the teacher to get a score (for completing the activity.) [The correct answers are in the "Downloads for Teachers" PDF.]

Note: the lab experiment can be done as a homework assignment as long as the student has access to the internet to watch the video.

If you want to actually perform the experiment in its entirety, a complete version of the lab experiment can be found on our website at http://www.rise.duke.edu.

Name _____Group #_____

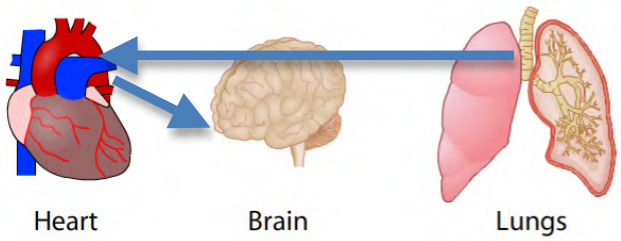
Mission #1

Answer the following questions:

1. Suppose Corey's dog ate a few cigarettes…do you think the nicotine in the cigarette could kill him (the dog)? ***YES***

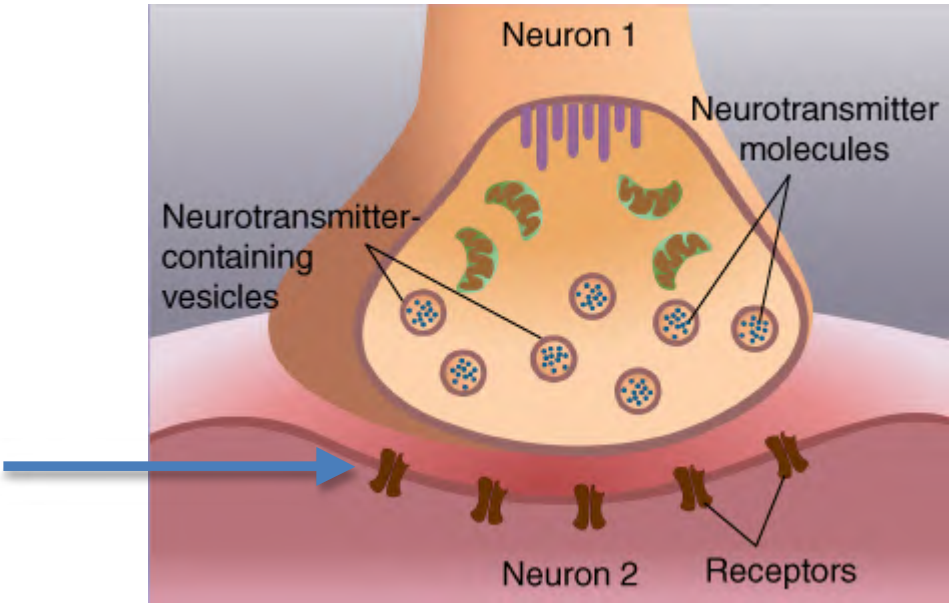
2. Corey's organs are shown below. Draw the path that the nicotine takes after Corey smokes a cigarette to get to the brain. Use arrows to designate the direction.

   Hint: Look at Figure 1.4 (or click the animation) to help you answer this question.



3. In the image below, draw an arrow to the structures at the synapse where the nicotine works.

   Hint: Look at Figures 1.7 and 1.8 (or click on the animations) to help you answer this question.



4. Which of the following effects is Corey likely to feel when nicotine is bound to receptors in his brain? Circle your answer.

   a. moody

   b. anxious

   c. alert

   d. depressed

5. Which of the following effects are produced when nicotine is bound to receptors in Corey's heart and blood vessels? Circle your answer(s).

   a. reduced heart rate

   b. increased heart rate

   c. reduced blood pressure

   d. increased blood pressure

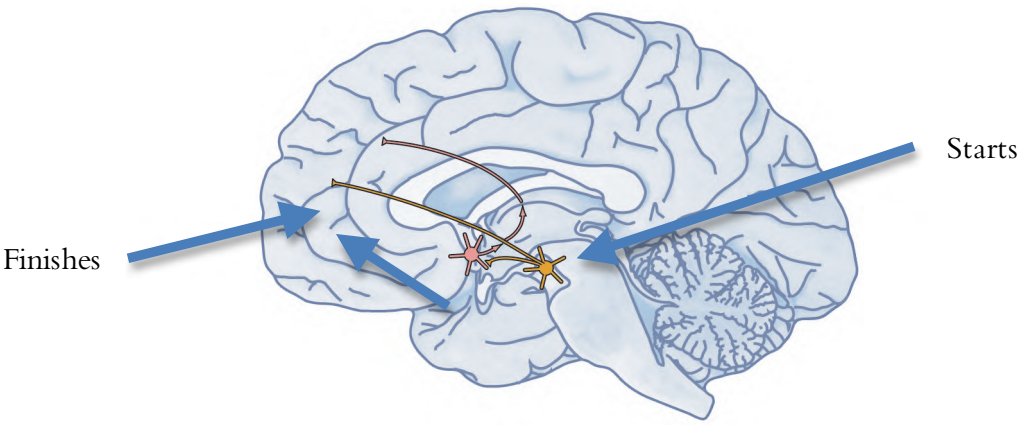
6. What is the main effect of nicotine on neurons?

   a. It increases the neuron's electrical activity

   b. It decreases the neuron's electrical activity

7. When Corey smokes, the nicotine stimulates activity of neurons along the reward pathway in his brain to increase feelings of pleasure (this explains why he likes smoking). Look at the picture of the brain and draw arrows along the reward pathway. Mark where it starts, and where it terminates.

   Hint: Look at Figure 1.9 to help you answer this question.



8. Corey's smoking over time caused several things to happen. Put a number next to the behavior in the order it occurs, and then draw a line to match the term with its definition.

_3_Addiction	Corey feels normal when he smokes; if he doesn't have a cigarette, he gets moody
_1_Tolerance	Corey needs more nicotine to get the same effect he got before
_2_Dependence	Corey can't stop smoking when he wants to; he has lost control over his own decisions

9. Circle the behaviors that are most likely associated with the increased number of nicotine receptors you saw in the smoker's (Corey's) PET scan on page 1.

Tolerance **Dependence** **Addiction**

10. Corey started smoking as an adolescent, at a time when the neurons in his brain were still forming their final synapses or connections.

What are the consequence(s) of this situation? Circle all that apply.

a. Corey is more likely to become addicted

b. Corey is more likely to become dependent

c. Corey is more likely to have trouble quitting smoking

d. Corey is more likely to try other addictive drugs

Name _____ Group #_____

Mission #2

Answer the following questions:

1. Tobacco contains many carcinogens (chemicals that cause cancer). Two of the major carcinogens in tobacco that cause cancer in humans are BaP and NNK. Match the 2 carcinogens with the way in which they are formed in the tobacco.

   BaP (benzo[a]pyrene) NNK

   When tobacco is "cured" or prepared for either smokeless or smoked forms, this chemical is produced from nicotine in the tobacco: **NNK**.

   As tobacco is burned, this chemical is produced as part of the combustion process: **BaP (benzo[a]pyrene)**.

2. It only takes one carcinogen to produce one mutation that can cause cancer. Name one gene that is mutated by carcinogens such as BaP leading to the development of cancer.

   p53

   What is the function of the protein that is synthesized under instructions of this gene?

   The p53 protein directs cells with damaged or mutated DNA to commit "suicide" or apoptosis.

3. Nicotine, in the absence of any tobacco, can also help tumors form. How can this happen? Circle your answer(s).

   a. Nicotine causes DNA damage

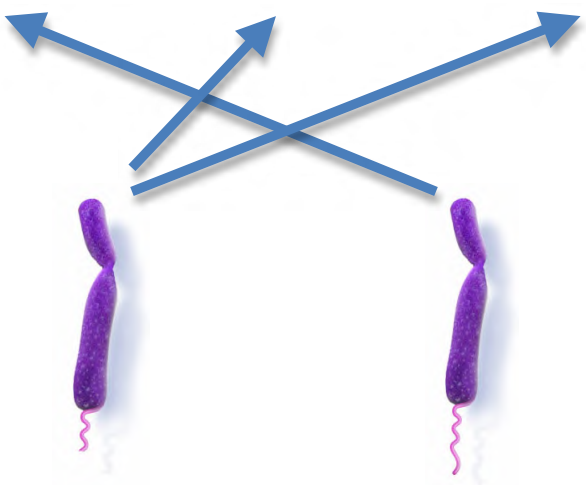
   b. Nicotine prevents chemotherapy drugs from killing cancer cells

   c. Nicotine causes tumor growth

   d. All of the above

4. Suppose people could get "telomere tested" when they go for their annual health checkup. They would give a cheek swab sample, and the lab would check the telomere length of their chromosomes. Can you match which chromosome is most likely found for each of the people below? (draw an arrow to connect the telomere with the people)

Young person **Smoker with cancer** **Old person**



5. It usually takes years to develop cancer after smoking or using smokeless tobacco. But how long does it take to form a mutation in DNA, which is one of the first steps in developing cancer? One way to find out is to participate in a real experiment. You can do this experiment in a laboratory or follow along with a video of other students performing the experiment in a lab.

   Before you start, indicate what you think the answers are to the following questions by checking the item:

   a. Can exposure to tobacco cause a mutation in DNA without smoking it?

      Yes____ No____

   b. How long would it take for tobacco to cause a mutation?

      Hours____ Days____ Months____ Years____

   Now, consult your lab packet to do the experiment—you can watch the video and then collect your own data. When you have finished, answer the following questions:

   a. Could the tobacco cause a mutation in DNA without smoking it?

      Yes____ No___

   b. How long did it take for tobacco to cause a mutation?

      Hours____ **Days**____ Months____ Years____

c. Do your answers agree or disagree with your prediction above?

Agree_____ Disagree_____

d. Now that you have determined how tobacco from one cigarette can cause a change in bacterial DNA, how could you test whether nicotine alone could cause a mutation in DNA? Use the lab activity you just saw in the video or in your Lab Activity packet to help guide your answer.

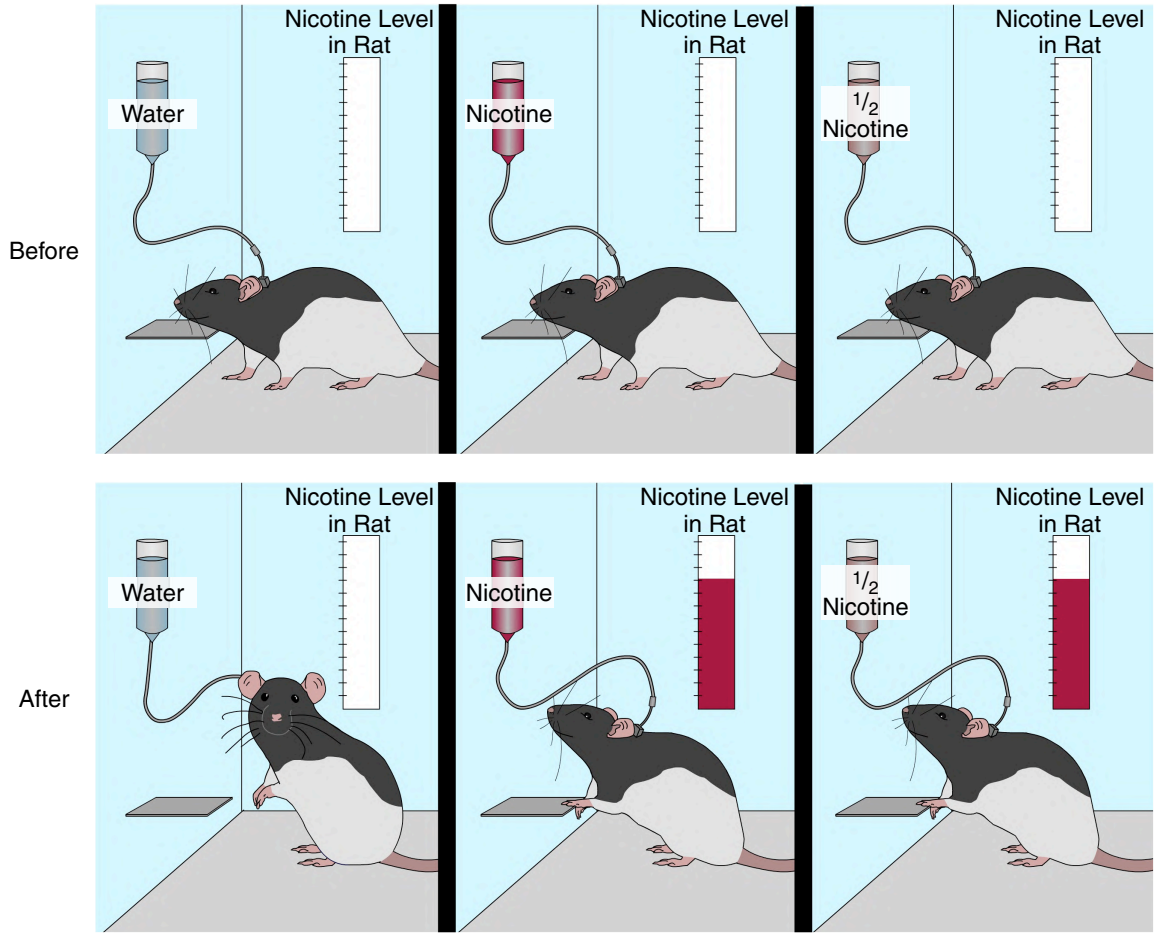
Get some pure nicotine and apply it to the Petri dish. Add the bacteria and let them grow for a few days. Look at the bacteria to see if any colonies turn white. This indicates that the nicotine produces DNA mutations in the bacteria.

Name _____ Group #_____

Mission #3

1. Animals such as rats enjoy nicotine just like humans. In experiments, rats will press a lever in a box to get an injection of nicotine. The pictures below shows a typical experiment. The 3 rats can press a lever to receive an injection of either A) water, B) water containing nicotine, or C) water containing half as much nicotine. After the experiment, in the lower panel, it shows how much nicotine each rat received.

   If you are viewing this page on a computer, **click on the top picture** to see how often each rat presses the lever, and how much nicotine each rat receives. You may have to watch the animation closely a few times to see what's happening.



   How much nicotine does the rat on the right get when it presses the lever for the bottle containing half the amount of nicotine? Circle your answer.

   a. half as much as the rat gets in the middle panel

   b. twice as much as the rat gets in the middle panel

   c. the same as the rat gets in the middle panel

How fast did the rat on the right press the lever to get nicotine?

a. half as fast as the rat pressed in the middle panel

b. twice as fast as the rat pressed in the middle panel

c. the same rate as the rat pressed in the middle panel

2. When Corey tried to cut down his smoking early on, he bought some "reduced-nicotine cigarettes". He thought this would help him quit.

   Do you think that he spent more or less money on cigarettes each week? _____

   Explain: He had to smoke more reduced-nicotine cigarettes to get the same amount of nicotine that he's used to getting, so he bought more.

3. From the list of chemicals below, write in which ones are found in each of the products shown in the table:

   Benzo[a]pyrene **Nicotine** **Nitrosamines** **Carbon Monoxide (CO)** **Tar**

   Hint: Be sure to consult your manual to determine how each of the products are formed (i.e. produced only when smoked, etc). You may need to refer to Module 2 to answer parts of this question.

Smokeless Tobacco	Reduced-Nicotine/Tar Cigarette	Snus	Regular Cigarette	Nicotine Patch	Tobacco Lozenge
Nicotine Nitrosamines	Benzo[a]pyrene Nicotine Nitrosamines CO Tar	Nicotine Nitrosamines	Benzo[a]pyrene Nicotine Nitrosamines CO Tar	Nicotine	Nicotine Nitrosamines

4. There is one ingredient in all the products listed above that is common to each. What is it? **Nicotine**.

   Explain why this ingredient must be present in any product to ensure that someone would want to use that product.

   Nicotine causes a pleasurable feeling, and helps the user want to use it again.

Give 2 reasons why this ingredient is harmful:

1. ***It is addictive***

2. ***It can promote cancer growth or it can cause high blood pressure/heart disease***

5. By now you have probably figured out why Corey received more carcinogens when he switched from smoking to smokeless tobacco. What could explain this?

He held the smokeless tobacco in his mouth for a long time, increasing his exposure to nitrosamines (NNK). Also, if there was less nicotine in the smokeless tobacco, he used more packets to get the level of nicotine he needed, exposing him to higher levels of carcinogens such as NNK.

Can Tobacco Cause Mutations in DNA?
A Virtual Experiment

Target Group: Health Education or Biology for Grades 9–10

SUMMARY

Objectives

The major objective in this experiment is to determine if compounds in tobacco can cause DNA mutations in a strain of bacteria (*Serratia marcescens*), in as little as 2 days. Mutations in bacterial DNA cause the bacteria to change from red to white. After completing the exercise, the students should be able to:

- Discuss the effect of tobacco on DNA
- Describe how a DNA mutation is manifested in *S. marcescens*
- Explain how fast a DNA mutation can occur
- Explain how one could determine if nicotine can cause mutations in the same system

HYPOTHESIS AND EXPERIMENTAL DESIGN

To address the objective of this exercise the following hypothesis can guide the experiment:

> *Compounds in tobacco will cause mutations in the DNA of the bacteria,* Serratia marcescens*, within 2 days.*

To test the hypothesis, one needs to design how to set up the experiment, including conditions that will serve as "controls". In this experiment, there are 3 experimental conditions to measure DNA mutations in *S. marcescens*: 1) extract of tobacco (in water), 2) UV light exposure, and 3) water alone. The water controls for possible mutations produced by something in the water that is used to make the extract. Second, the UV light causes mutations in many organisms, including humans. It is used to show that the experiment actually works…the UV light should cause DNA mutations regardless of what happens with the tobacco condition.

MATERIALS

- Online access to the 10 minute "virtual lab", performed by peer students (www.rise.duke.edu/seek).
- Color printouts of the 3 agar plates containing bacterial colonies—enough for one set of images to each group of students (see Appendix).

METHODS

Serratia Marcescens is a strain of bacteria commonly found in the environment. *S. Marcescens* produces a red pigment (i.e. colonies are red) when grown at 24-30° C. Mutations in the bacterial DNA for the red pigment will cause the colonies to turn white (they don't make the red pigment) or pink (they make less of the red pigment).

UV light is used as a control condition to show that the experiment works. UV radiation causes mutations in the bacterial DNA and the mutated colonies should turn white (UV light will also kill some of the colonies).

Watch the video

What's not in the video: The preparation

Prior to the actual experiment shown in the video, the teacher carried out some preparation. The preparation included the following:

- Make stock plates of bacteria by inoculating agar (nutrients in gelatin) plates with the bacteria purchased from a biological company
- Store the plates for 2 days at room temperature (24-25°C)
- Transfer a single colony of bacteria to a tube containing liquid nutrients (nutrient broth)
- Store the liquid bacteria culture for 1 day before starting the experiment
- Before starting the experiment, dilute the bacteria (1 drop) in a new tube containing 10 milliliters of fresh nutrient broth. Then repeat. This dilutes the bacteria to be used for the experiment.

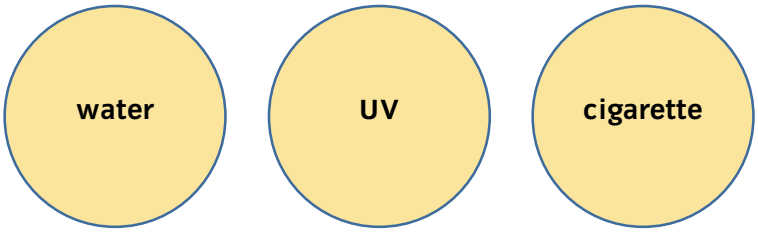
What's in the video: Experiment day

Step 1: Students make the tobacco extracts

- Heat water, cut open a cigarette and put tobacco in the water, stirring for 5 minutes
- Heat another beaker of water for the control
- Filter the extract through cheesecloth into a bottle

Step 2: Students add tobacco extracts to plates

- Label the underside of 3 agar plates as follows:



- Place 1 drop of the following solutions in the middle of each agar plate

  Water plate: add previously heated water

  UV plate: add previously heated water

  Cigarette plate: add cigarette extract (in water)

A student might ask why you would add the water to the UV plate, and the reason is that with this design all 3 plates contain the same water, providing a fair comparison.

- Use glass beads to roll the solution all over the plate, let solution dry for ~20 minutes

Step 3: Add bacteria to the plates

- Place 1 drop of bacterial solution into the middle of each plate
- Spread the bacteria on the plate using fresh glass beads; let dry for ~ 10 minutes
- For the UV plate, place the UV lamp over a box with a hole in the top and shine the UV light through the hole onto the plate for 15–20 seconds. This should cause mutations in the bacterial DNA. It will also kill many bacterial cells.
- Store the bacterial plates for 2 days at room temperature
- Take pictures of the plates and send to a computer

Collect Data: Done in class, this is no longer virtual

Data collection is done 2 days after the bacteria were treated; students will count the number of mutated bacterial colonies in each plate that was prepared by the students in the video to determine mutations.

- The teacher should hand out color copies of the 3 plates, one set for each group (see below). Images of the plates are found in the appendix if additional copies are needed.

- The plates are already coded as follows:

  Plate A = UV; B = cigarette; C = water control

- Students should indicate on their lab datasheet what they expect to find BEFORE looking at the plates. Ask the students to circle the level of mutations they expect for each plate:

Water plate	No mutations	A few mutations	A lot of mutations
UV exposed plate	No mutations	A few mutations	A lot of mutations
Cigarette-extract plate	No mutations	A few mutations	A lot of mutations

- Teacher hands out pictures of Plates A, B, and C, to each group; explain that these are the actual results from the experiment that the students did in the video

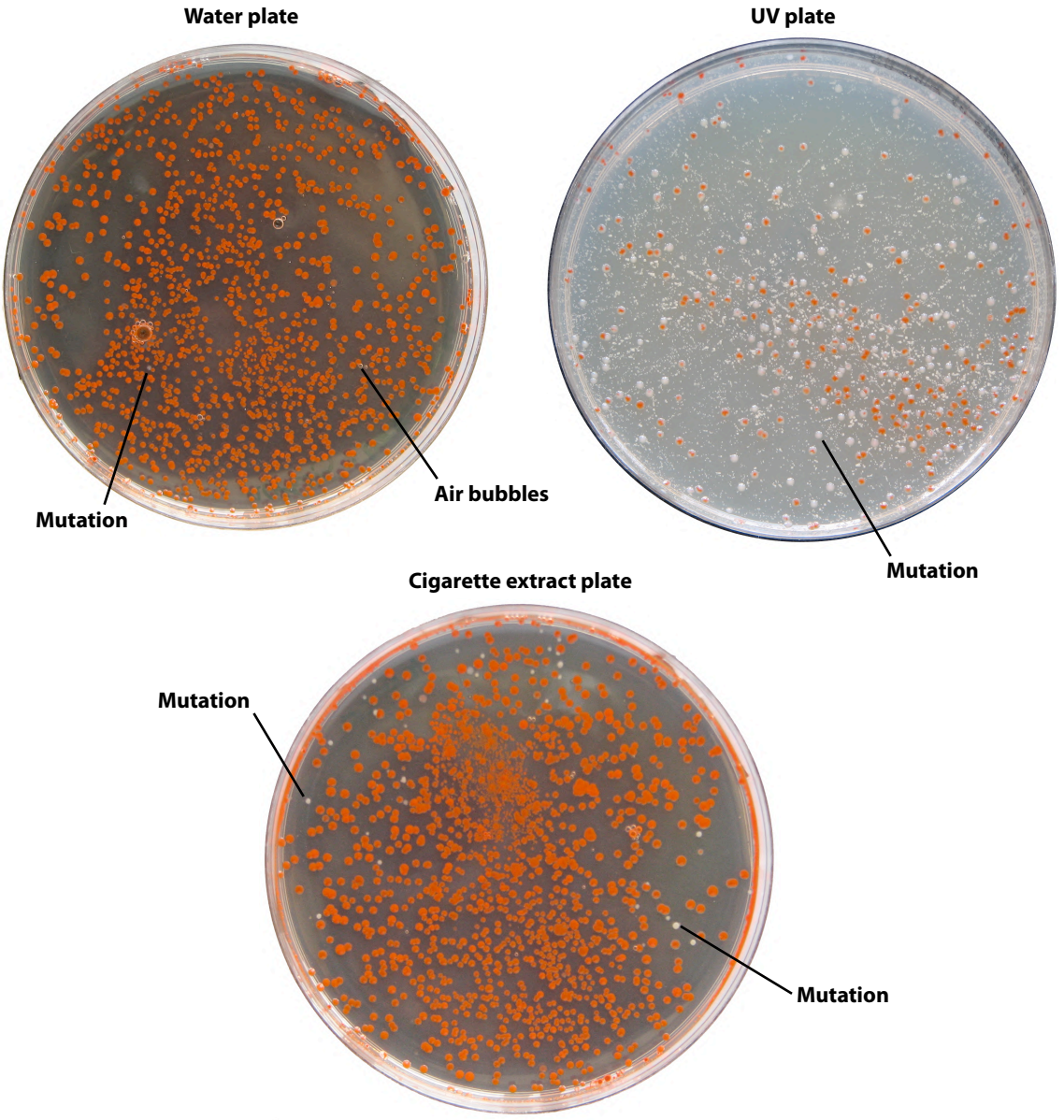
- Explain to the class that the plates are coded so that no bias will be introduced when counting colonies. The students should not know the treatment groups of the plates until after the data are collected.

- Students count the number of white spots on the printout of each plate.

red = normal DNA white = mutated DNA

To count most efficiently, have students draw a line through each white spot as they count so that spots are not counted twice.

Sample images of the plates



- Students should record their bacteria counts in the following table that is found in the student lab datasheet. They should get similar data to that shown in the table:

Plate Treatment	# of white colonies on plate
Plate A	~100
Plate B	30
Plate C	1

- After the students have recorded their data in the table, "break the code"—tell them which plate is which. Have them write the actual treatment in the table under the code letters. (A = UV exposed; B = cigarette; C = water)
- Have students answer the questions on their datasheets so that a discussion about the experiment can be conducted.

REFERENCE

This activity is modified from the original activity developed by Rebecca Milholland, Graduate Student & NSF CATTS Fellow, Department of Pharmacology & Toxicology, University of Arizona, and Stefani D. Hines, M.A., M.S., Director Community Outreach and Education Program, Southwest Environmental Health Sciences Center, University of Arizona.

APPENDIX

1. Student data sheets (answers are filled in)
2. Agar plate images (coded A, B, and C)

STUDENT DATA SHEET **Name:** _____

Before you start, what do you expect to see? Circle the level of mutations you expect for each plate:

Water plate	No mutations	A few mutations	A lot of mutations
UV-exposed plate	No mutations	A few mutations	A lot of mutations
Cigarette extract plate	No mutations	A few mutations	A lot of mutations

---

Now count the number of white spots on each plate. It might be easier to draw a line through each white colony as you count.

Record your data in the following table:

Plate Treatment	# of white colonies on plate
Plate A	
Plate B	
Plate C	

Remember: red = normal DNA white = mutated DNA

Based on your results, what do you think was added to each plate (water, UV light, or cigarette extract)?

Plate A: _____Plate B: _____Plate C: _____

Explain in 2–3 sentences why you chose your answers.

Now, ask your teacher to identify the conditions for each plate. Write them into the table in the first column. Did you guess correctly which plate was which? If not, what are some reasons why your answers were wrong?

Some possible answers:

Water had more mutations in it than expected

UV light did not mutate all of the bacterial colonies

Tobacco extract did not mutate all of the bacterial colonies

Go back and look at your predictions (before you counted colonies) for the level of DNA mutations produced in each condition. Which of your predictions were not supported by the results of the experiment?

What are some reasons why the experimental results did not support your predictions?

Possible answers:

Perhaps some background mutations in bacterial DNA are normal,

Colonies were difficult to count giving errors

Didn't think that 2 days was long enough for tobacco to mutate DNA

Agar Plate Images for Printing and Counting

Print a copy of each plate in color and give to students to count colonies.

Code **(FOR TEACHER'S EYES ONLY!!)**

Plate A = UV

Plate B = cigarette extract

Plate C = water

Plate A

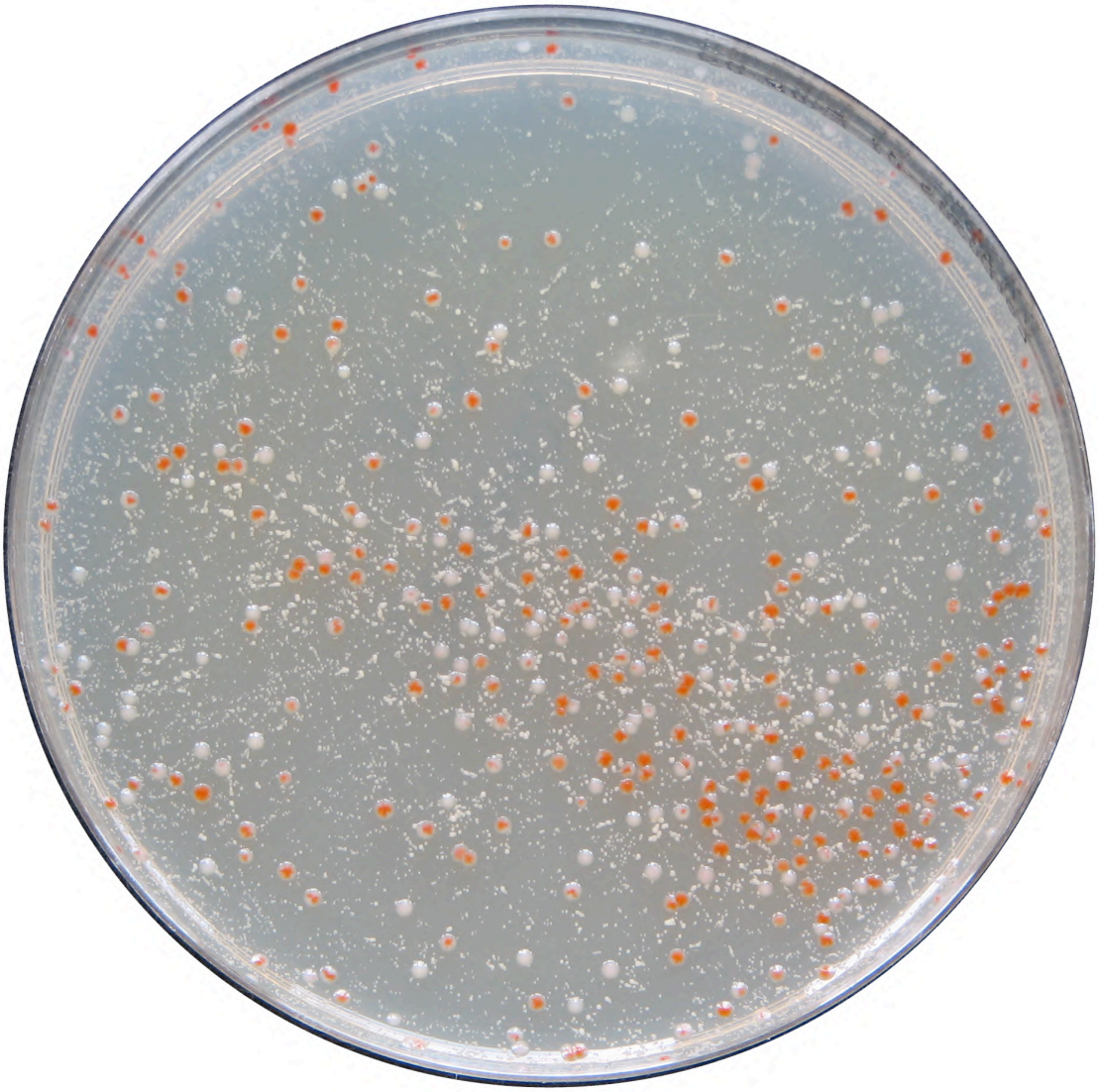


Plate B

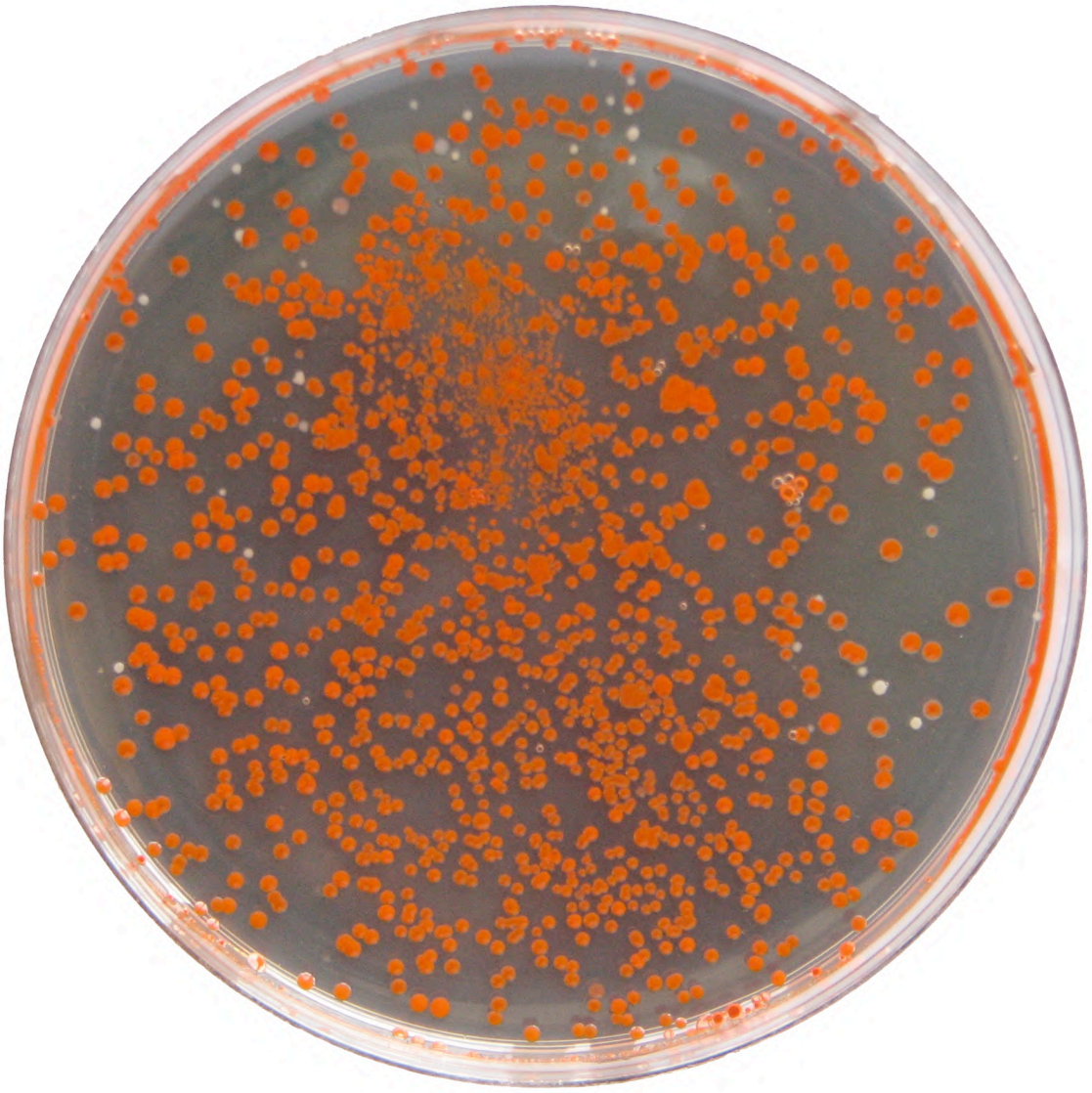


Plate C