pep Pharmacology Education Partnership

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How do drugs damage neurons? It's radical!

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A Project Funded by a NIDA Science Education Drug Abuse Partnership Award

Module 3: How do drugs damage neurons? It's radical!

Description of the module

This module focuses on the chemical process of oxidation and reduction initiated by drugs such as methamphetamine. The oxidation causes the formation of free radicals which damage specific neurons in the brain. In this unit, the role of oxygen in oxidative reactions within the body to maintain normal function and to promote cell damage is presented. The concept of oxygen radicals is introduced and the ability of oxygen radicals to attack (oxidize) cellular lipids, proteins and DNA is described. The exercise demonstrates how oxidation of these cellular components initiated by drugs such as methamphetamine causes the cells to degenerate. Similar reactions occur in response to normal aging, radiation and in neurodegenerative diseases.

Learning objectives

After participating in this module, students should understand the following:

1. The properties of a compound that make it oxidizable
2. How oxygen participates in an oxidative reaction
3. How a compound becomes reduced
4. Why neurons with oxidizable neurotransmitters are most susceptible to damage
5. How an oxygen radical is formed
6. How oxygen radicals oxidize substrates such as proteins, lipids and DNA
7. The role of enzymes in oxidative reactions
8. How oxidation of cellular components produces toxicity

This module integrates information from the following areas:

chemistry, neurobiology, cell biology, biochemistry

Student Handout

Drugs such as methamphetamine ("speed") interact with specific neurons in the brain to produce their stimulant effects. Methamphetamine and other derivatives of amphetamine are known to produce damage to certain neurons in the brain. This was recently shown in the brains of long-term human users of methamphetamine. To understand how this happens, we must understand how methamphetamine interacts with the brain. Methamphetamine increases the release of the neurotransmitter dopamine from specific neurons where it is stored in vesicles. The extra dopamine binds to dopamine receptors on neurons and causes activation of the central nervous system to increase alertness and cause hyperactivity. Normally dopamine action is terminated when it is transported back into neurons. There, it is oxidized by oxygen (O_2) with the help of enzymes (oxidases) in the mitochondria.

1. Create a drawing of a neuron and indicate where the dopamine is stored and where the oxidases are found.

2. What kinds of components are necessary for a compound to be oxidized?

3. How does the chemical structure of dopamine enable it to become oxidized?

4. Show a simple reaction equation for the oxidation of dopamine and describe what happens. Indicate which compound becomes oxidized and which compound becomes reduced. Explain what happens to electrons in this process.

5. What kinds of products are formed once dopamine becomes oxidized?

Dopamine undergoes a different fate during methamphetamine exposure. When methamphetamine is present, dopamine accumulates in the space between the neurons and can be oxidized by O_2 in the absence of help from enzymes. This is called "autooxidation". One of the products formed is called an oxygen radical. Oxygen radicals, like free radicals, are highly reactive and unstable molecules.

6. Show a simple reaction for the autooxidation of dopamine and describe what happens.

7. What kind of oxygen radical is formed once dopamine is autooxidized?

8. Describe the structure of a radical. What makes a radical so unstable?

Normally the oxygen radical formed by autooxidation of dopamine is destroyed (scavenged) by special enzymes that change them into hydrogen peroxide (H_2O_2) and then into water. However, if the concentration of H_2O_2 becomes too high, the scavengers are overpowered. In the presence of ferrous ion (Fe^{2+}), the H_2O_2 is reduced to form even more reactive oxygen radicals which then attack (oxidize) cellular components such as proteins, lipids and DNA.

9. How does Fe^{2+} reduce H_2O_2? What kind of oxygen radical is formed?

10. Where in the neurons are the proteins, lipids and DNA found primarily?

11. What is the chemical nature of proteins, lipids and DNA that enables them to be attacked (oxidized) by radicals?

When proteins, lipids and DNA are oxidized by the radicals they become damaged. This affects their ability to perform their normal functions. Ultimately, the cell membranes become leaky and the cells then degenerate.

12. What are the primary functions of proteins, lipids and DNA inside cells?

13. Once the cell membranes become leaky, what makes them die?

Once specific neurons have become damaged, the normal function of the part of the brain in which they reside is disrupted. For example, neurons containing dopamine in a part of the brain called the caudate nucleus are important in controlling movement. After repeated use of drugs such as methamphetamine, movement disorders can develop. This is very similar to what happens in Parkinson's Disease, which involves oxidative damage to dopamine neurons in the caudate nucleus.

14. List 4 additional examples of oxidative cellular damage to specific targets within the body by drugs and disease.

Anatomy of the dopamine neuron

Neurons are the principal cells of the brain; they comprise a cell body (or soma), dendrites and an axon that ends at a terminal (see **Figure 1**). The cell body contains the nucleus where DNA resides along with the nuclear machinery to direct the synthesis of proteins. The cell body is also the part of the cell in which an electrical impulse is generated. The dendrites form a branching structure off of the cell body and act as receivers. Neurotransmitters and drugs bind to receptors on dendrites that trigger membrane currents to promote or inhibit the electrical impulse generated in the cell body. The electrical impulse travels down the axon to the terminal where neurotransmitters are synthesized, stored and then released.

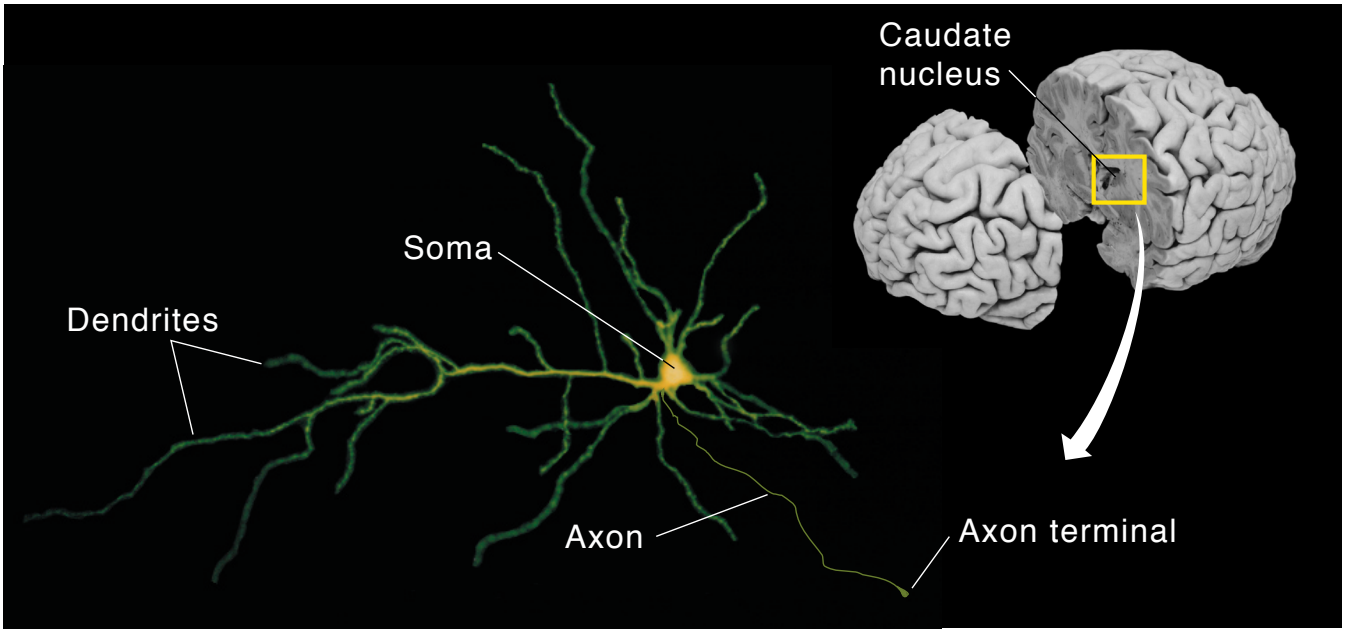


Figure 1. A real neuron filled with a yellow dye shows its structure. A major group of neurons that contain dopamine send their axons into the caudate nucleus, an area of the brain involved in emotion and movement.

Neurons synthesize specific chemicals called neurotransmitters. These neurotransmitters are stored in small sacs, or vesicles, in the axon terminal and they are released from the terminal following the arrival of an electrical impulse. For example, dopamine neurons synthesize dopamine by a series of enzymatic reactions that takes place in the neuron terminal. When an electrical impulse originating in the cell body travels down the axon to the terminal, it triggers the release of dopamine from the vesicles into the space between neurons (the synapse) (**Figure 2**). Dopamine binds to dopamine receptors on neighboring dendrites to alter membrane currents.

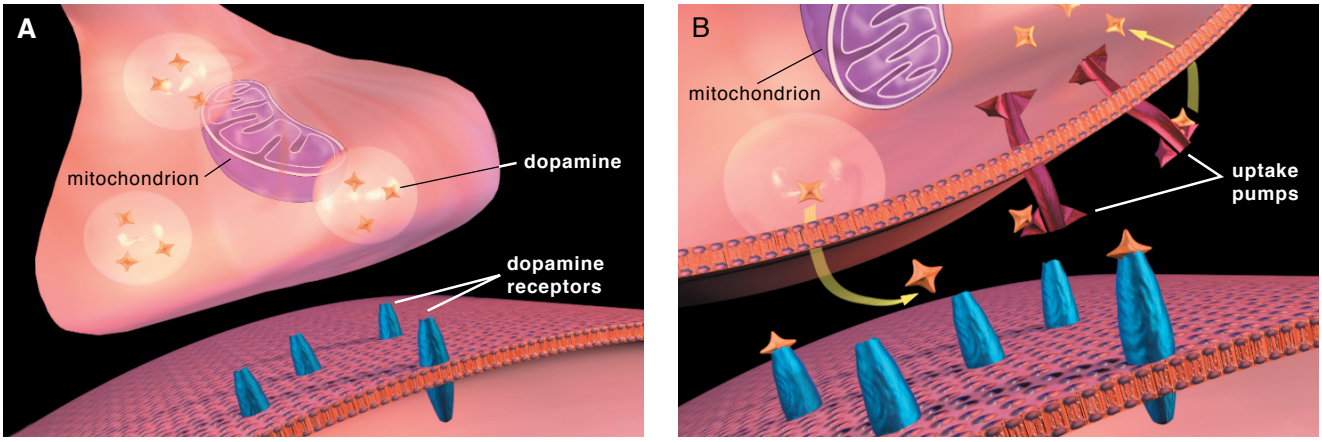


Figure 2. *Left:* Axon terminals release dopamine into the synaptic space. Dopamine binds to dopamine receptors. *Right:* Dopamine is removed from the synaptic space by uptake pumps (also called transporters).

Oxidation of dopamine

Once dopamine is released into the synaptic space, its actions are terminated in two ways. First, it is transported back into the dopamine terminal by "uptake pumps" (**Figure 2**), which are special proteins that act as transporters. Second, dopamine is oxidized by oxygen (O_2), which is available after it diffuses from blood capillaries into spaces surrounding the neurons. The dopamine can be oxidized by two different processes. Normally, oxidation of dopamine by O_2 is catalyzed by enzymes called monoamine oxidases. These oxidases are found in the mitochondria within the nerve terminal. Dopamine is a monoamine; it contains one amine (NH_2) group. Its structure is shown in **Figure 3**. So, once inside the terminal, the monoamine oxidase helps O_2 remove an electron (associated with the H) from the CH_2 group nearest the NH_2 on dopamine. The loss of the e- from the CH_2 group removes the amine group (NH_2) as well (the H combines with NH_2 to form NH_3 or ammonia). Now, one O atom can bind to fill its octet with a double bond to CH. This general reaction for the oxidation of dopamine is shown in **Figure 3**.

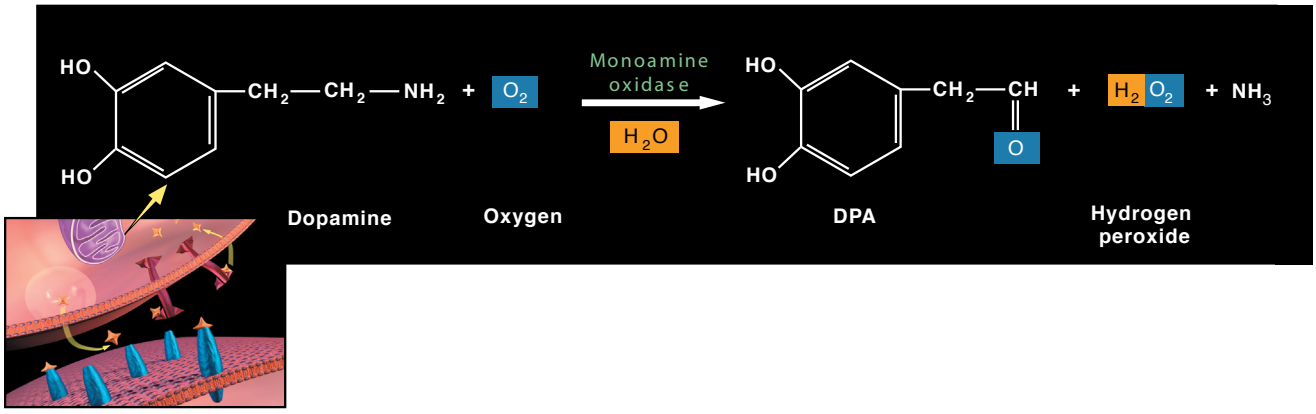


Figure 3. Inside the terminal, dopamine is oxidized by O_2 with the help of the mitochondrial enzyme monoamine oxidase to form DPA, a metabolite, and hydrogen peroxide.

Molecular oxygen (O_2) contains 12 electrons in the outer orbital, but 2 of these electrons are unpaired. Because of the 2 unpaired electrons in the outer orbital, O_2 likes to accept a single electron from compounds such as dopamine or metals in the body (e.g., iron, copper); it is a good oxidizing agent for this reason. Thus, the dopamine becomes oxidized and the O_2 becomes reduced. In other words, O_2 acts as an oxidizing agent to oxidize dopamine; dopamine acts as a reducing agent to reduce O_2. This oxidative reaction results in the formation of a metabolite called dihydroxyphenylacetaldehyde (DPA), an inactive neurotransmitter metabolite. The other O atom combines with H_2O to form hydrogen peroxide (H_2O_2), which is converted back to water with the help of another enzyme called catalase.

Interaction of methamphetamine with dopamine neurons: Autooxidation

There are times when dopamine concentrations rise in the synaptic space (or even inside the terminal). When this happens, the dopamine can be oxidized by O_2 without the help of any enzyme. This is called "autooxidation". The drug methamphetamine is a good example to describe how autooxidation of dopamine gets started and how cells undergo oxidative damage. Methamphetamine, like amphetamine, is a central nervous system stimulant. It causes the release of dopamine from the axon terminal into the space between the neurons (the synaptic space). The excess dopamine in the synaptic space binds to dopamine receptors on specific neurons to cause stimulation of certain pathways in the brain, resulting in increased alertness and agitation. With repeated use of methamphetamine or when high doses are used, dopamine accumulates in the synaptic space because the uptake pumps become saturated; they are not able to transport the dopamine back inside the terminal very efficiently. The excess dopamine then becomes autooxidized by O_2 present in the extracellular space, without the help of any enzymes. However, the autooxidation of dopamine occurs at the other end of the molecule, compared to enzyme-catalyzed oxidation. The reaction for autooxidation of dopamine is shown in **Figure 4**.

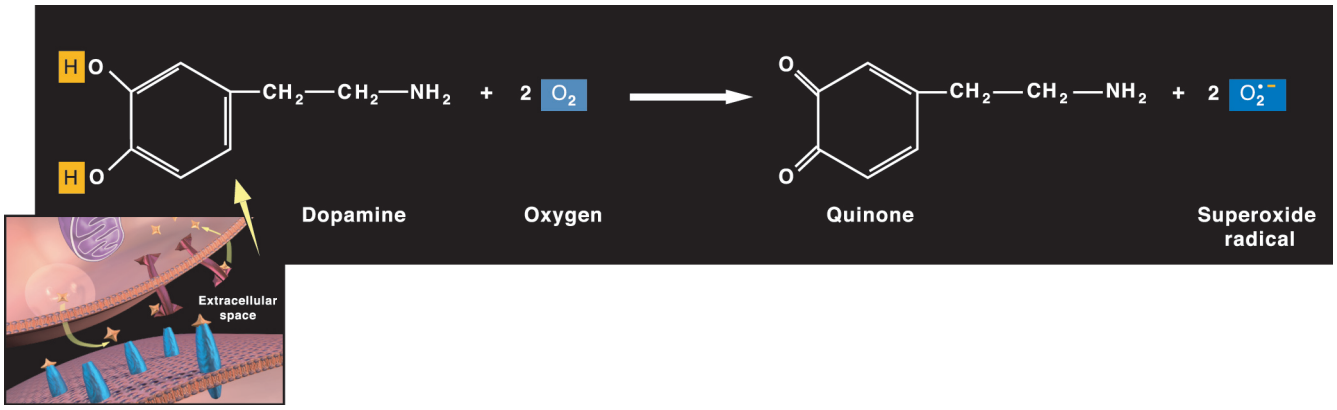


Figure 4. Dopamine is oxidized by O_2 alone to form a quinone and the superoxide radical. This can occur in the synaptic space or inside the terminal.

Production of oxygen radicals by methamphetamine

During the autooxidation of dopamine, dopamine loses an e- from each of the two OH groups on the ring to form a compound called a "quinone" (see **Figure 4**). Oxygen picks up the e- (2 molecules of O_2 picked up an e- each) leaving it with an extra unpaired e- (it now has 13 e- instead of 12 in the outer orbital). This is a very unstable form of oxygen called a superoxide radical (O_2•-). This oxygen radical is a type of free radical. Free radicals have a lone electron in their outer electron orbital (**Figure 5**) and they are very reactive molecules because they tend to donate a single e- or steal one from another

molecule. Free radicals often have a • shown to indicate the lone e-.

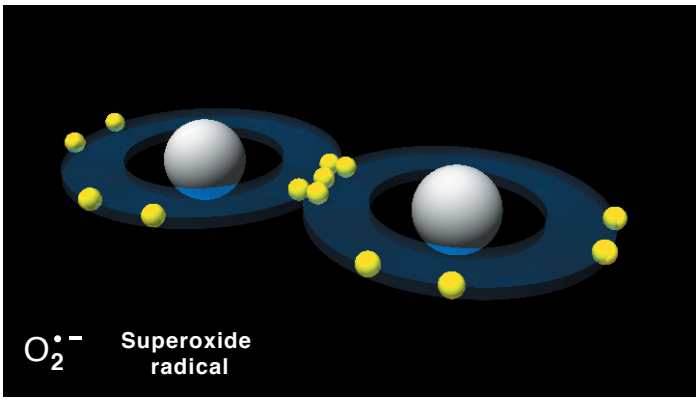


Figure 5. The superoxide radical contains 13 electrons in the outer orbitals of the 2 oxygen atoms. The extra electron gives the molecule a very reactive character.

To keep the level of oxygen radicals low in our bodies, oxygen radicals are "scavenged" by enzymes to render them harmless. For example, superoxide radicals are reduced with the help of the enzyme superoxide dismutase to form hydrogen peroxide (H_2O_2), which is then converted (detoxified) by the enzyme catalase to water and O_2 (see Figure 6 for the entire process). However, levels of oxygen radicals can rise, for example, after repeated or excessive use of drugs such as methamphetamine, or during aging, when scavengers may be not as active. In this case, hydrogen peroxide becomes reduced by iron (Fe^{2+}), which donates an electron, to produce the hydroxyl radical (HO•), a very nasty molecule. It is extremely reactive, and it's a great oxidizing agent. The hydroxyl radical oxidizes cellular components such as lipids, proteins and DNA by literally stealing an e- (associated with an H atom) from them. This damages cells (see below).

Most recently, newer theories to explain the neurotoxicityof methamphetamine have emerged. These involve effects of methamphetamine on the dopamine transporter to disrupt ion homeostatis. This will not be discussed here.

Cellular targets for oxidation by oxygen radicals

Lipids, proteins and DNA are especially good targets for oxidation by oxygen radicals. The location of lipids, proteins and DNA in the cell is crucial for cell function. For example, the lipids are found primarily in the plasma and nuclear membranes (and membranes of other cellular organelles), where they provide the structural matrix for the membrane. Proteins are found scattered about within the membrane and in the cytoplasm, where they perform numerous functions. They provide structural support within the cell, they function as enzymes and as receptors, and they provide the machinery for cells to undergo respiration. DNA is found in the nucleus in the form of chromosomes and it provides the starting material to synthesize proteins.

Lipids, proteins and DNA all contain H atoms bound to C atoms that are easily stolen by the hydroxyl radical (OH•). Hydroxyl radicals steal H atoms from the C chains of lipids and amino acids (the smallest unit that forms proteins), and from DNA bases. Many proteins also contain sulfur atoms bound to H atoms. When the OH• steals an H from the sulfur groups (i.e. oxidizes the SH group), disulfide bonds (S—S) are formed. As the lipids, proteins and DNA become oxidized by the OH•, they become radicals themselves (i.e., they contain lone e- within their structure), creating a vicious cycle for radical generation (see **Figure 7**).

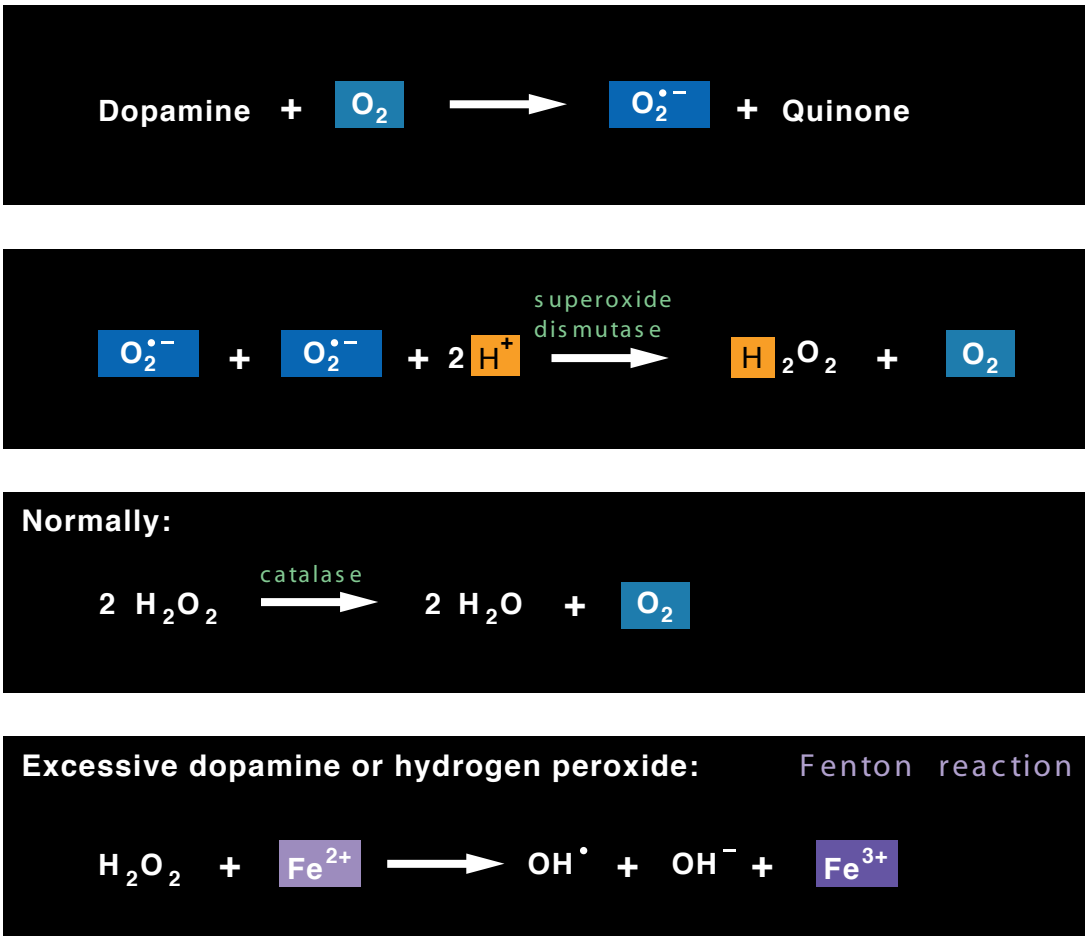


Figure 6. Dopamine is oxidized by oxygen sequentially to form the superoxide radical, hydrogen peroxide and the hydroxyl radical. The hydroxyl radical is the most reactive and most damaging form of oxygen radicals.

Cell death by radical attack

Oxidation of lipids, proteins and DNA by oxygen radicals (and by other free radicals that are formed in the process) causes their structure to become disorganized (**Figure 7**). Cross-linking of lipids, proteins and DNA occurs and polymers start to form. This causes major problems. Damaged proteins cannot perform their normal cellular functions and damaged DNA cannot participate in the synthesis of new proteins required to keep the cell alive. In the case of lipids, their carbon chains become truncated and "kinky" due to the loss of H atoms. Thus, the lipids no longer form a tight cellular membrane barrier and the cells and organelles become leaky. Water can enter the cells causing them to swell (edema); they die eventually by bursting. This process can take a very short time, for example, during radiation, or it can take a long time, such as after repeated use of drugs like methamphetamine, or even during the aging process.

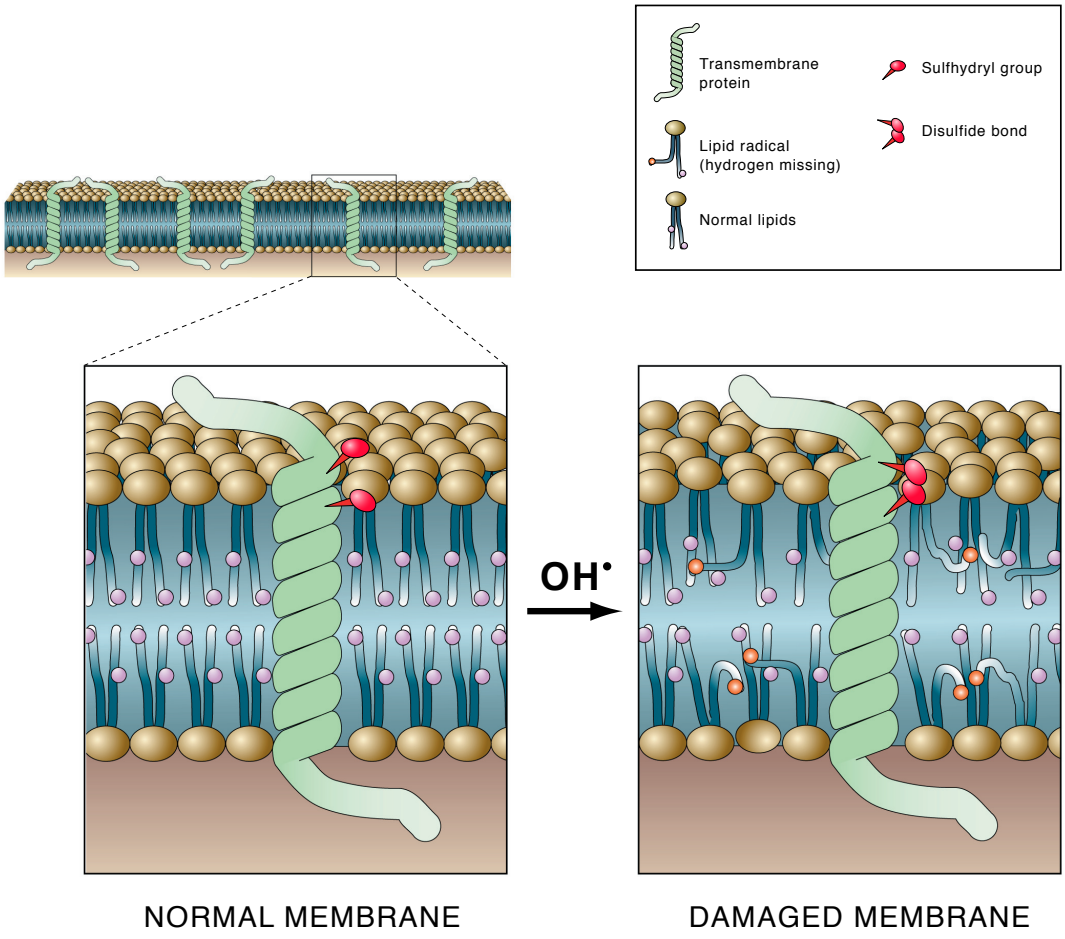


Figure 7. Hydroxyl radicals (OH●) damage membrane lipids and proteins by removing H atoms from the lipid chains and from protein sulfhydryl groups (SH). The structure of both lipids and proteins is disturbed,

Effects of oxidative damage to specific neuron targets by drugs and disease

Some neurons in the brain are more susceptible to oxidative damage than others. Those neurons that contain dopamine and other oxidizable neurotransmitters, such as serotonin, are quite vulnerable (as explained above). For example, methylenedioxy-methamphetamine ("MDMA" or "ecstacy") is another amphetamine-like drug that produces oxidative damage to serotonin neurons. While such damage has been shown in laboratory animals including primates, it is only recently that the neurotoxic effects of methamphetamine has been shown in the brains of human abusers. This is most likely due to the recent resurgence of methamphetamine abuse.

Oxidative damage (caused by any means) in different parts of the brain can produce different neurological effects. For example, dopamine neurons are found in pathways that control voluntary movement. Oxidative damage to dopamine neurons in these pathways can cause movement problems. Similarly, movement disorders are associated with Parkinson's disease and Huntington's disease, in which dopamine neurons degenerate within an area of the brain called the basal ganglia. The basal ganglia

are important in maintaining voluntary movement; degeneration within these structures results in tremors and tics. A few years ago scientists discovered an impurity (MPTP) in synthetic heroin that destroys dopamine neurons. It caused Parkinson's disease in young men that were using the heroin. Another disease, Alzheimer's Disease, involves the loss of function in the hippocampus, an area of the brain involved in memory. Oxidative damage to neurons in the hippocampus contributes to the degeneration in Alzheimer's disease. Other examples of oxidative damage to cells include, 1) radiation damage to specific targets (accidental or for cancer treatment, or even UV rays from sunlight), 2) damage to tumor cells by various anti-cancer drugs that form radicals easily, 3) damage to motor neurons in the brain and spinal cord due to a loss of the scavenging enzyme, superoxide dismutase in Lou Gherig's disease (Amyotrophic Lateral Sclerosis or ALS), 4) damage to the liver and other organs by radicals formed from long-term alcohol use and 5) degeneration of many kinds of cells during normal aging, when levels of oxygen radical scavenging enzymes decrease.

Glossary

dopamine—a neurotransmitter stored in vesicles of nerve terminals; it is a monoamine that is easily oxidized. This neurotransmitter is contained in neuron pathways important in brain stimulation, addiction and control of movement.

enzyme—a protein that catalyzes the rate at which a reaction occurs. It binds to one of the reactants (a substrate) to cause a change in the reactant's structure, facilitating the reaction.

free radical—an atomic or molecular species in which 1 or more outer orbital(s) contains an unpaired (lone) electron.

hydrogen peroxide (H_2O_2)—formed from the reduction of the superoxide radical by superoxide dismutase, but it is not actually a radical. It is detoxified by conversion to O_2 and H_2O by the enzyme catalase.

methamphetamine ("speed")—a derivative of amphetamine that increases the release of dopamine into the synaptic space. It causes increased alertness, restlessness and agitation.

monoamine oxidase—the mitochondrial enzyme that helps O_2 oxidize monoamines such as dopamine to inert products. Monoamines have one amine group (NH_2) within the molecule.

molecular oxygen (O_2)—the form of oxygen in air and in the blood. It consists of 2 oxygen atoms, each having 6 electrons. The 2 atoms share a pair of electrons to form a bond, leaving 1 unpaired electron for each atom in their outer orbital. It likes to accept an electron from many kinds of donors (becomes reduced).

oxidation—the donation of electrons to another atom often by removal of a H+ atom.

oxidizing agent—a species that is good at gaining electrons (e.g. O_2, Fe^{3+})

oxygen radicals—the reduction of oxygen by sequentially gaining electrons yields oxygen radicals such as the superoxide ($O_2\bullet$-) and the hydroxyl (OH•) radical. It is the OH• that damages lipids, proteins and DNA.

reduction—the gain of electrons from another atom, often by addition of a H+ atom.

reducing agent—a species that is good at donating electrons (e.g. Fe^{2+})

serotonin—a neurotransmitter that is also oxidized easily by oxygen. Serotonin neurons are damaged by another amphetamine-like drug, methylenedioxy-methamphetamine (MDMA or ecstacy).

superoxide dismutase—the enzyme that reduces the superoxide radical ($O_2\bullet$-) to hydrogen peroxide (H_2O_2). This enzyme diminishes with aging and in some neurodegenerative disorders like Alzheimer's disease.

synapse—the connection between two neurons; neurotransmitters are released from the terminal into the synaptic space and bind to receptors on the neighboring neuron.

Module 3: Supplemental Classroom Activities

"An Apple Without A Peel"

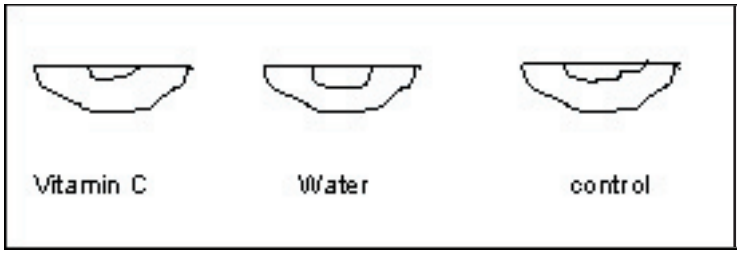
Objective(s):
1. To demonstrate how an apple becomes oxidized in air and understand the process of oxidation.
2. To investigate the action of an anti-oxidant on the browning process of an apple.
3. To design and conduct scientific investigation.

Standards and Skills:
AA1, AA2, AA3, AD2, AE1, CB2, CB3, CE2, CF1, CF4, CF5, CG1, CG2, CG3

Science Concepts:
Once the skin of the apple is removed, the exposure of apple to the air will result in oxidation of the tissues of the apple. Browning is due to polyphenoloxidase, an enzyme that causes the tannins to condense and change color. Inside the apple the polyphenol and the polyphenoloxidase are separate. Chilling to 4º C inhibits the reaction. Boiling denatures (destroys the structure and function of) the enzyme. The chloride ion will inhibit the reaction. In acidic solutions such as lemon juice, the enzyme works slowly. Ascorbic acid (vitamin C), a known anti-oxidant, can prevent oxidation.

Procedure:

Set-up
1. Crush a vitamin C tablet and dissolve in 100 mL of distilled water in a beaker labeled "A".
2. Pour 100 mL of distilled water in a beaker labeled "B".
3. Cut three identical wedges about 1/2 cm thick from an apple. Include the heart of the apple. **Immediately** after cutting, place one wedge in solution "A" and one wedge in solution "B". Make sure all surfaces of the wedges are wet with the solution.
4. Remove the wedges from the beakers and place all three wedges on a tray appropriately labeled.



Assignment
Working in student groups of four, design an experiment based to test on of the following hypothesis or a hypothesis of your own:

Hypothesis:
1. The greater the concentration of the ascorbic acid the longer the apple will be protected from browning time.
2. Different varieties of apples will be affected differently.
3. _____(select another fruit) will not be preserved from browning.
4. Vitamin E, another antioxidant, will work better than vitamin C.

5. Not allowing the cut apple to be exposed to oxygen will prevent the browning.
6. The rate of browning will increase as the temperature increases.
7. Lowering the pH prevents the browning.
8. Any acid will prevent the browning of the apple.
9. Browning only occurs on the surface of the apple.
10. _____ others

Data: Record the time required for the first browning to occur. Record the comparative brown for 24 hours. Sketch your sample and describe where browning occurs.

Results:

Discussion of Results:

Follow-up:
Questions for discussion:
1. Why doesn't applesauce turn brown?
2. What could be another control for the teacher's demonstration?
3. Why don't the vegetables in a commercially packaged ready to eat salad turn brown?
4. What characteristic of the apple skin prevents browning of the apple?
5. What characteristics would you genetically engineer into the apple to inhibit the browning effect?
6. If apples normally contain vitamin C, why does it not prevent the browning?
7. Do oranges undergo browning? Why or why not?
8. Why were the English sailors called "limeys"?
9. What is the RDA for vitamin C?

Recommendations for Assessment:
1. Evaluation of lab reports that includes experimental design, data, and conclusions.
2. Answers to questions.
3. Conduct a class seminar - The Prevention of Browning in Apples. Have each group present their experimental results and conclude with a discussion of how some fruits can be preserved and how this lab relates to unit 3 and the oxidation processes occurring in the body.

Teacher Notes:
This activity can be done as a student designed lab or a teacher demonstration.

Use this opportunity to give an "A" to an investigation in which the students get data to reject a hypothesis. This should be the case in some of the hypotheses listed.

Fruit should be as fresh as possible. Old apples do **NOT** work very well.

Other fruits tested were bananas, peaches, pears, and potatoes. The apple, pear and potato work best. The banana peel turns brown almost immediately. This was slowed by vitamin C but difficult to see any difference after a few minutes.

Vitamin E is a fat-soluble vitamin and can be purchased in capsules of oil or dry. The dry form is not very water-soluble. Neither worked very well. The control vegetable oil and the oil form of Vitamin E were about the same. The active ingredient of vitamin E here is in the form of di-alpha tocopheral acetate. This ester must be hydrolyzed in the stomach to become active. Its lack of hydrolysis probably

explains its ineffectiveness on the apple. Di-alpha tocopherols are commonly found in fish oils, vegetable oils, and wheat germ.

Immediately covering with plastic wrap did not protect from browning.

Research indicates that a 0.5% pectin solution will prevent the browning in fruits. Analysis shows the active ingredient that prevents browning is not the pectin but small amounts of oxalic acid present in the commercial pectin preparation. Pure oxalic acid in not an approved food additive but it does occur naturally in fruits and vegetables, especially rhubarb and spinach.

Interesting tid-bit:
Albert Szent-Gyorgyi, a Hungarian biochemist, became interested in plant chemistry when he saw a similarity between the darkening color of damaged fruit and that of patients suffering from adrenal gland disorders. He isolated the substance that delayed browning in plants for analysis. This is how ascorbic acid, vitamin C, was first discovered.

Websites:
www.nysaes.cornell.edu/fst/ Describes instruments used to quantify color change in fruits.

Books:
Enzymatic Browning and its Prevention. ACS Symposium Series 600
ISBN 0-8412-3249-0

Harold McGee, *On Food and Cooking - The Science and Lore of the Kitchen*, Collier Books, Macmillian Publishing Co, New York

Resources

The following resources provide supplemental information that pertains to the topic in this module.

RR Levine, CA Walsh and RD Schwartz. *Pharmacology: Drug Actions and Reactions*, Parthenon Publishing Group, New York, 2000. Chapters 4-6, 15.

SH Snyder, *Drugs and the Brain* , WH Freeman and Co. New York, 1996. Chapter 1.

BA Freeman and JD Crapo (1982). *Biology of Disease. Free Radicals and Tissue Injury.* Laboratory Investigation 47: 412-426.

MJ De Vito and GC Wagner (1989). *Methamphetamine-induced neuronal damage: A possible role for free radicals.* Neuropharmacology 28:1145-1150.