

SEADAP LESSON OUTLINE

Lesson	Title	Description
1	Acute Activity (Stereotypy)	C-shapes increase during acute drug exposure
2	Repeated Activity (Stereotypy)	Tolerance or sensitization to C-shapes occurs during repeated drug exposure
3	Acute Activity (Motility)	Locomotion (grid crossings) changes during acute drug exposure
4	Natural Anxiety	Greater time in dark indicates greater anxiety
5	Dependence (Anxiety and Drug Withdrawal)	Greater time in dark following drug withdrawal indicates greater anxiety
6	Dependence (Depression and Drug Withdrawal)	Decreased motility/activity following drug withdrawal indicates depression
7	Reward and Reinforcement (Conditioned Place Preference)	Environmental cues associated with drug reward facilitate drug addiction
8	Drug-Drug Interactions	Combinations of caffeine/sugar, caffeine/alcohol, etc. can produce additive, synergistic or sub-additive effects on activity and reward

Teacher's Guide: Acute Sterotypy--50 minutes total

Goal: To demonstrate that drugs of abuse affect activity following acute exposure. Stimulants such as nicotine and caffeine will produce increased activity following acute exposure and this effect in humans is due to increased dopamine transmission in area of the brain called the limbic system. The C-shapes observed in this experiment represent a stereotyped behavior. Stereotyped behaviors are defined as repetitive, meaningless movements and are commonly observed when stimulants are administered to rodents.

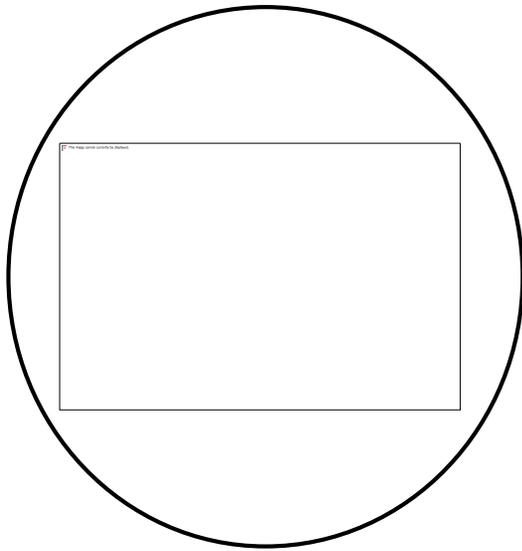
Part 1: Vocabulary--approximately 5-10 minutes

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Concentration	The amount of dissolved solute per unit volume of solution
Acute	A response of rapid onset and/or short duration
Stereotypy	Repetitive or ritualistic movement (Ex: Body rocking, C-Shaped Movements)

Part 2: Observations --approximately 10 minutes

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope as well as list your observations.

Under observations students should note color, movement, size, shape, etc. It is recommended that the microscopes are set up for the students.



Part 3: Experiment-20-25 minutes (including group sharing of results)

How is a planarian affected by nicotine, caffeine, alcohol, and sucrose? Note: each group will be assigned one of the above substances to investigate

Break the class into four groups and assign each group to one of the four conditions (nicotine, caffeine, alcohol, or sucrose). This portion should take 20 minutes (4 concentrations x 5 minutes per concentration). Have the students share their results with other groups (e.g. via white board) before moving onto the discussion questions.

NICOTINE

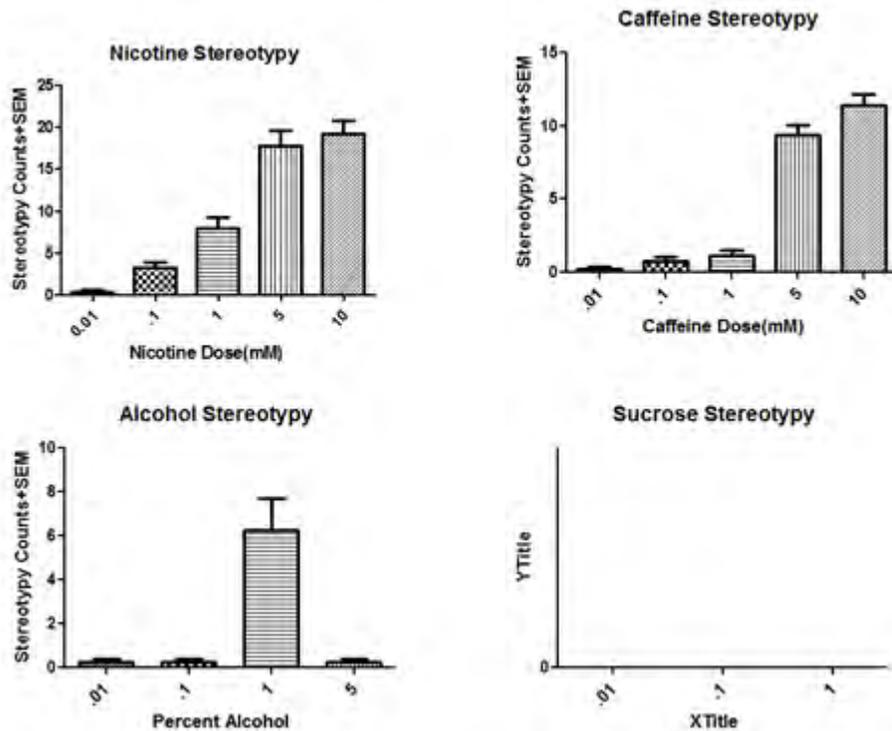
1. Label a petri dish with “.01 mM Nicotine”.
2. Place your planarian on a petri dish with .01 mM of Nicotine. **Make sure your students label the petri dishes to keep them straight!**
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian in a petri dish with spring water. **The amount of time you choose to leave the planarian in the spring water between trials can vary according to the time constraints of your classroom. This experiment can be done entirely in one class period, or over the course of numerous days depending on what suits your needs best.**
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Nicotine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. **Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose.**

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	4	8	18	22
Trial 2				
Trial 3				
Average (Trials 1-3)				

**Note: these are the values obtained with our own experimental procedure, they may vary slightly from classroom to classroom. It is recommended that you test these on your own first!

Fill in the graph with your results below (use the averages).



CAFFEINE

1. Label a petri dish with “.01 mM Caffeine”.
2. Place your planarian on a petri dish with .01 mM of Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place the planarian back in a petri dish of spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Caffeine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. **Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose.**

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	2	3	9	13
Trial 2				
Trial 3				
Average (Trials 1-3)				

****Again, these values are results obtained for our own experimental procedure and will vary!**

Fill in the graph with your results below. **Example graphs for each substance can be found above**

ALCOHOL

1. Label a petri dish with “.01 mM Caffeine”.
2. Place your planarian on a petri dish with .01 mM of Alcohol.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place the planarian back in a petri dish of spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Alcohol concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. **Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose.**

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	1	8	1	1
Trial 2				
Trial 3				
Average (Trials 1-3)				

****Note, results may vary**

Fill in the graph with your results below. [Example graphs for each substance can be found above](#)

SUCROSE

1. Label a petri dish with “.01 mM Caffeine”.
2. Place your planarian on a petri dish with .01 mM of Sucrose.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place the planarian back in a petri dish of spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Sucrose concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	0	0	0	0
Trial 2	0	0	0	0
Trial 3	0	0	0	0
Average (Trials 1-3)	0	0	0	0

****Sucrose is the control condition**

Fill in the graph with your results below. [Example graphs for each substance can be found above](#)

Part 4: Scientific Method Recap--approximately 10 minutes

What **question** were we trying to answer with this experiment?

[What happens to planarians with increasing drug concentrations?](#)

What might have been our **hypothesis**?

[The more drug you give a planarian, the greater the number of C-Shaped curves you will observe](#)

[*Note: a valid hypothesis might also predict that there would be no effect of drug concentrations on movement](#)

What **methods** did we use to test our hypothesis?

Placing planarians into four different concentrations of the drug solution and counting the number of times they exhibited the C-shape movement in a five minute interval

What were our **results**?

For both **Nicotine** and **Caffeine**, increased substance concentration is associated with increased movement (C-Shaped Curves)

For **Alcohol** there is a sharp increase in movement at 1 mM but the effect most disappears with a smaller or larger concentration.

Sucrose is the control condition and as a result there was no effect of increasing concentration on movement.

What **conclusions** can we draw from our results?

Nicotine and **caffeine** exposure will increase movement

Alcohol will increase movement at a certain concentration (1 mM) but have little/no effect at other concentrations

Sucrose exposure have no effect on movement

Part 5: Discussion Questions--approximately 10 minutes

1. What patterns do you notice as the concentrations increase?
Generally, as concentrations increase, there is increased movement (Note: Sucrose is a control so no changes are observed in this condition)
2. Is there a relationship between substance concentration and planarian movement?
For every substance but sucrose, generally the higher the concentration, the greater the movement
3. How can you tell that an increased substance concentration is having a greater effect on the planarian?
You can tell that increasing substance concentration is having a greater effect on the planarian because the planarian moves more with higher concentrations. This might suggest that these substances have the potential to have physical effects on the organisms that ingest them.
4. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how these substances (nicotine, caffeine, alcohol, etc.) affect our own bodies?

If these substances are having physical effects on planarians, then they probably will have physical effects on humans as well. Also, in general, the more of a substance you ingest, the greater the physical effects will be.

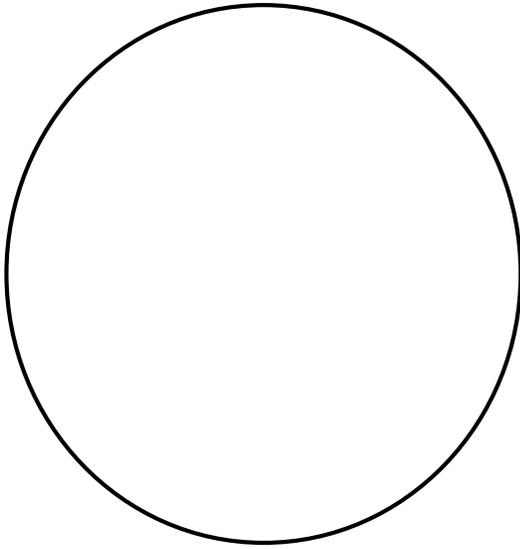
Acute Stereotypy

Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
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Acute	A response of rapid onset and/or short duration
Stereotypy	Repetitive or ritualistic movement (Ex: Body rocking, C-Shaped Movements)

Part 2: Observation

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope as well as list your observations.



Part 3: Experiment

How is a planarian affected by nicotine, caffeine, alcohol, and sucrose? Note: each group will be assigned one of the above substances to investigate

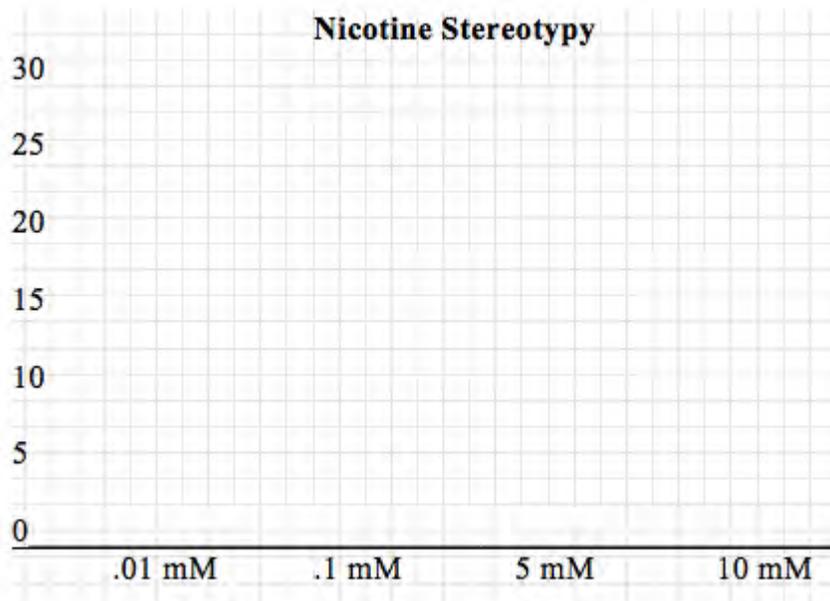
NICOTINE

1. Label a petri dish with “.01 mM Nicotine”.
2. Place your planarian on a petri dish with .01 mM of Nicotine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian in a petri dish with spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Nicotine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1				
Trial 2				
Trial 3				
Average (Trials 1-3)				

Fill in the graph with your results below (use the averages!).



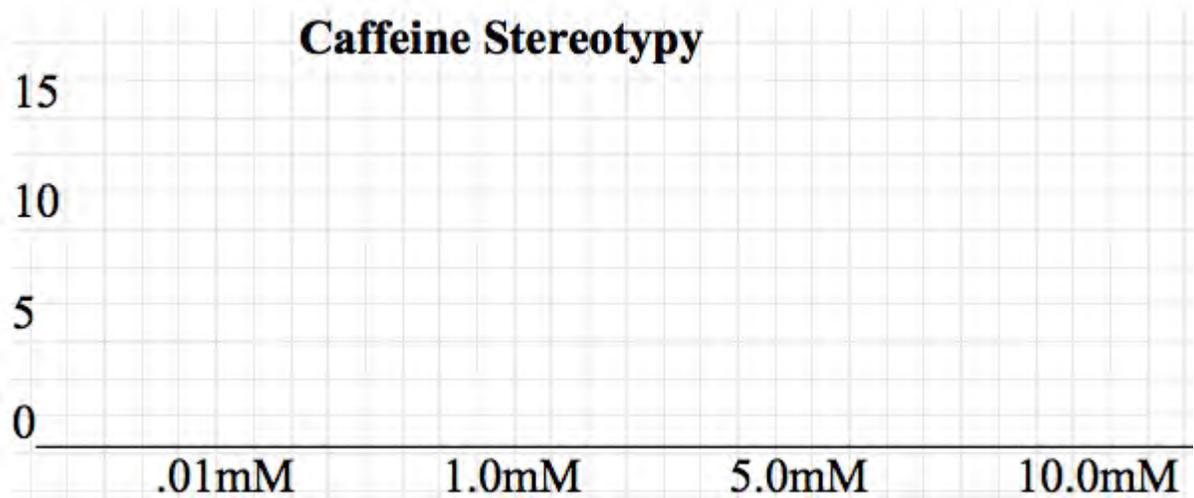
CAFFEINE

1. Label a petri dish with “.01 mM Caffeine”.
2. Place your planarian on a petri dish with .01 mM of Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back in a petri dish with Spring Water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Caffeine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1				
Trial 2				
Trial 3				
Average (Trials 1-3)				

Fill in the graph with your results below (use the averages!).



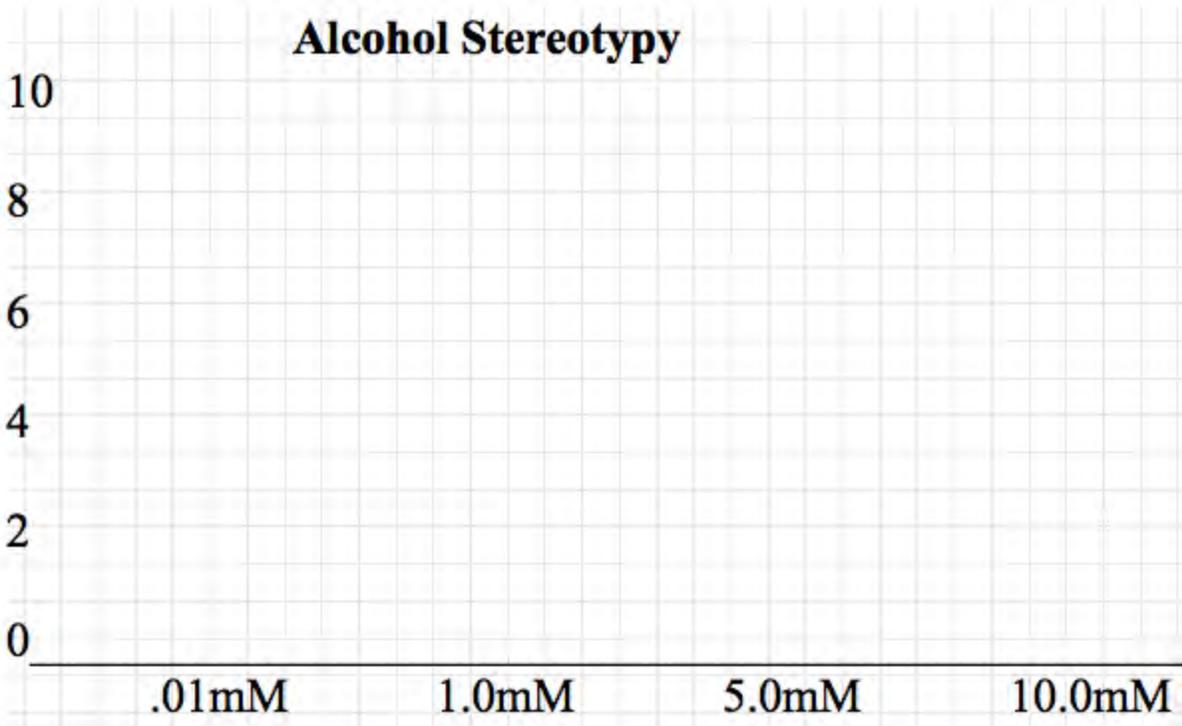
ALCOHOL

1. Label a petri dish with “.01 mM Alcohol”
2. Place your planarian on a petri dish with .01 mM of Alcohol.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Alcohol concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1				
Trial 2				
Trial 3				
Average (Trials 1-3)				

Fill in the graph with your results below (use the averages!).



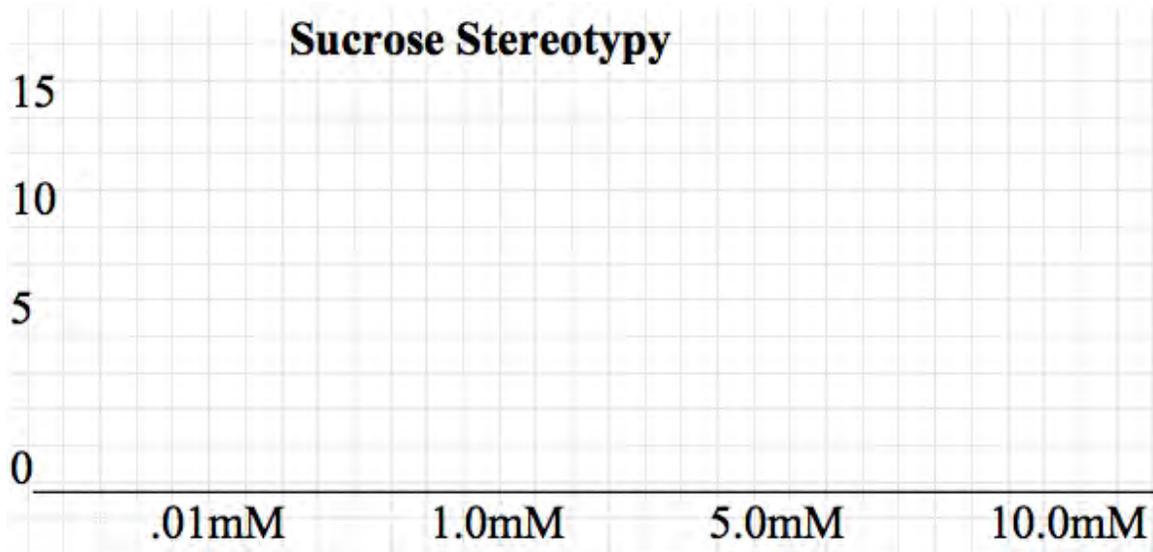
SUCROSE

1. Label a petri dish “.01 mM Sucrose”
2. Place your planarian on a petri dish with .01 mM of Sucrose.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 1-4 an additional 2 times with different planarians.

Repeat the above with Sucrose concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1				
Trial 2				
Trial 3				
Average (Trials 1-3)				

Fill in the graph with your results below (use the averages!).



Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. What patterns do you notice as the concentrations increase?

2. Is there a relationship between drug concentration and planarian movement?

3. How can you tell that an increased drug concentration is having a greater effect on the planarian?

4. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how these substances (nicotine, caffeine, alcohol, etc.) affect our own bodies?

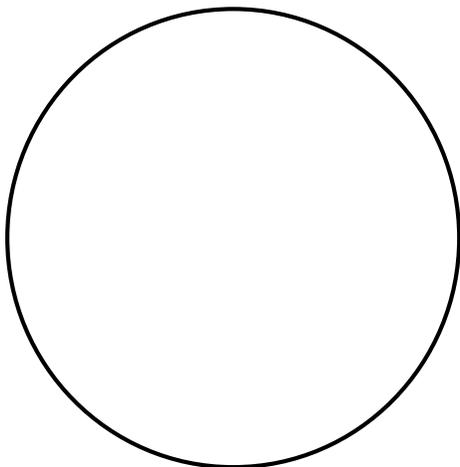
Lesson 2: Repeated Stereotypy

Goal: To demonstrate using planarians that drugs of abuse produce changes in activity during repeated exposure. Repeated exposure to a substance may produce a diminished biological response, which is defined as tolerance, or an augmented biological response, which is defined as sensitization. Repeated exposure is also defined as chronic exposure. The pattern of chronic exposure can mimic addictive use in humans (i.e., binge-like administration on a daily basis) or recreation use (i.e., periodic use that may be only on the weekends or once a month).

Part 1: Vocabulary

Planarian	A kind of flatworm, and the subject of this experiment
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Concentration	The amount of dissolved solute per unit volume of solution
Stereotypy	Repetitive or ritualistic movement (Ex: Body rocking, C-Shaped Movements)
Tolerance	a drug or concentration of drug loses its effectiveness with chronic exposure
Sensitization	an exaggerated response during chronic exposure that sometimes occurs with drugs of abuse
Dependence	the state of being psychologically or physiologically dependent on a drug after a prolonged period of use
Physical Dependence	dependence that involves persistent physical–somatic withdrawal symptoms (e.g., fatigue and delirium tremens)
Psychological Dependence	dependence that involves emotional–motivational withdrawal symptoms (e.g., dysphoria and anhedonia)

Part 2: Observation



1. Examine the planarian under a microscope and draw what you see. List your observations below.

Under observations students should note color, movement, size, shape, etc.

It is recommended that the microscopes are set up for the students.

Part 3: Experiment

How is a planarian affected by repeated exposure to nicotine, caffeine, and alcohol?

Note: each group will be assigned one of the above substances to investigate

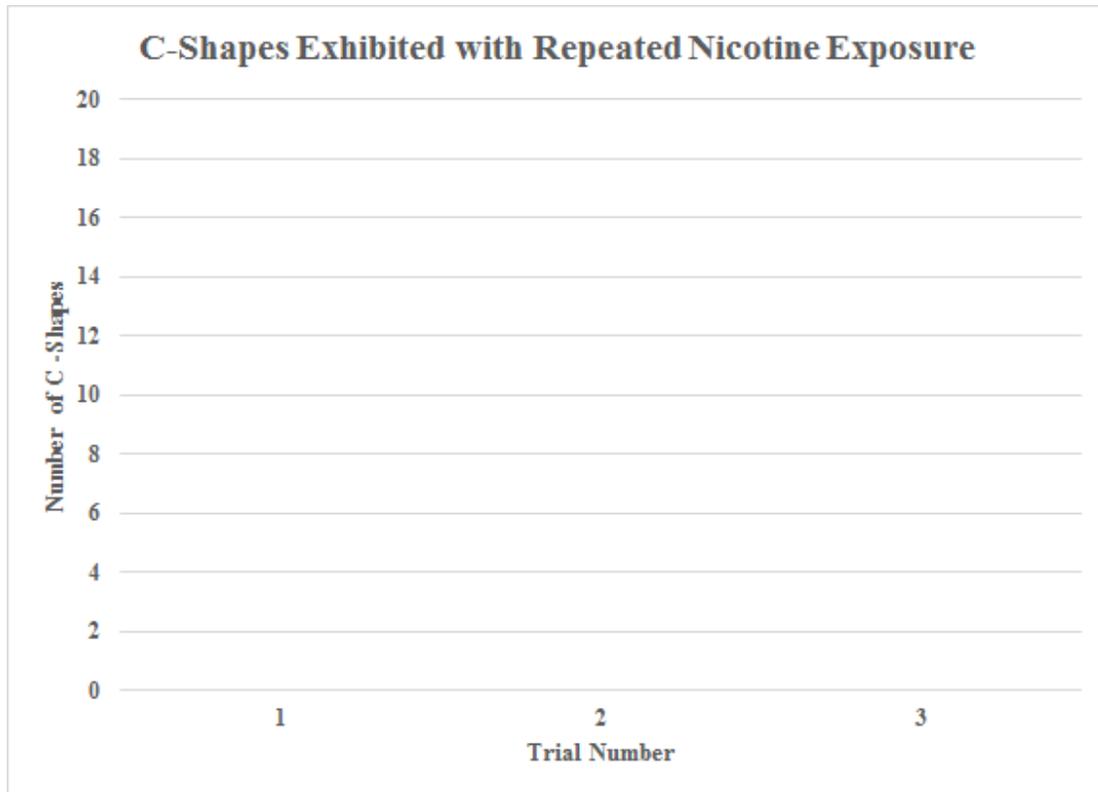
This experiment is testing sensitization versus tolerance. It is very hard to predict which substances will result in either scenario, as it will differ from planarian to planarian. Sensitization will occur when the response becomes more and more robust with additional trials, and tolerance occurs when the response becomes smaller and smaller with additional trials. Both sensitization and tolerance occur as a result of chronic exposure, which is why three trials are necessary for each planarian tested.

You can also vary the number of times the planarians are exposed to the drugs or the duration of the withdrawal interval. For example, you might try exposing the planarians to the drug for 3 straight days using a withdrawal interval of 24 hours. The long withdrawal interval would mimic recreational drug use in a human whereas the shorter withdrawal intervals of 8 minutes would mimic a binge-type exposure that is characteristic of addictive drug abuse.

NICOTINE

1. Prepare one petri dish with a 1 mM solution of nicotine and one petri dish filled with spring water and place both on a plain white piece of paper. **You will want to test out the drug concentration under your own laboratory conditions. We recommend starting with 1 mM. You may need to adjust the concentration slightly down (e.g. 0.1 mM) if the planarians die with repeated exposure or slightly up (e.g. 5 mM) if the response is not robust enough.**
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the nicotine solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph with your results below

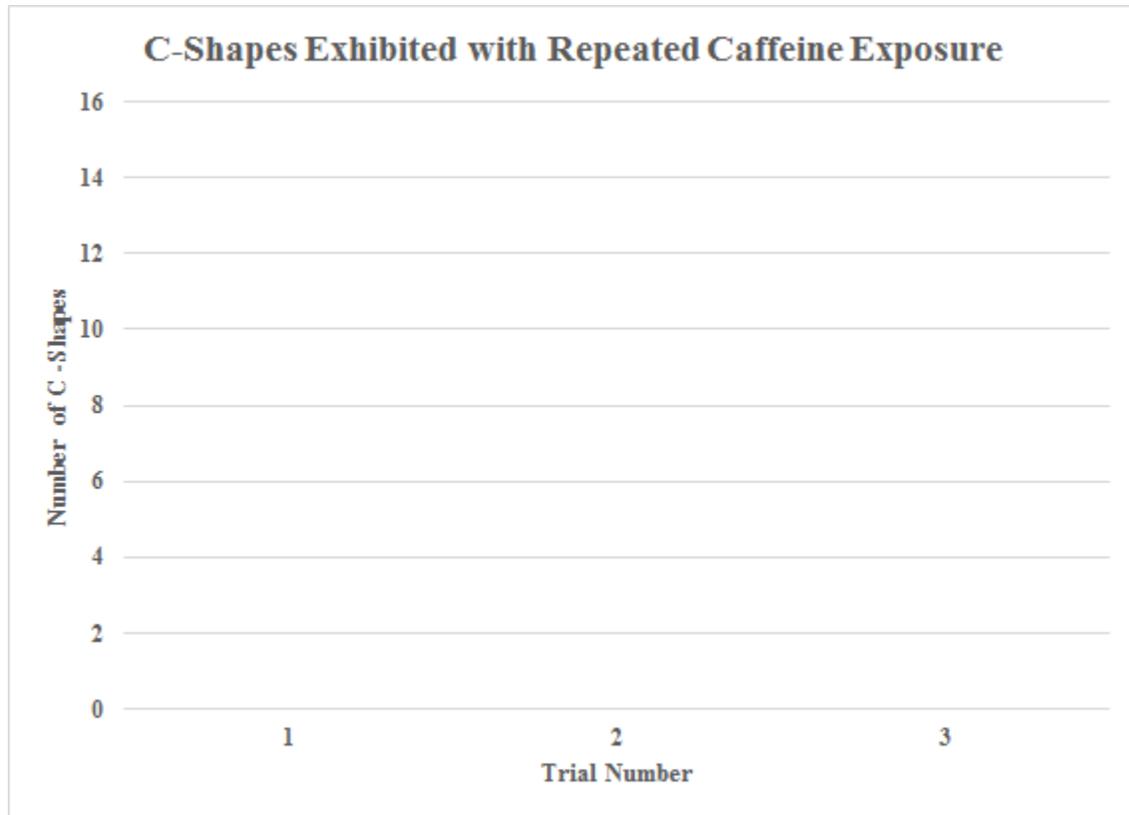
Trial	1	2	3
# of C-shapes			



CAFFEINE

1. Prepare one petri dish with a 1 mM solution of caffeine and one petri dish filled with spring water and place both on a plain white piece of paper. **You will want to test out the drug concentration under your own laboratory conditions. We recommend starting with 1 mM. You may need to adjust the concentration slightly down (e.g. 0.1 mM) if the planarians die with repeated exposure or slightly up (e.g. 5 mM) if the response is not robust enough.**
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the caffeine solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph with your results below

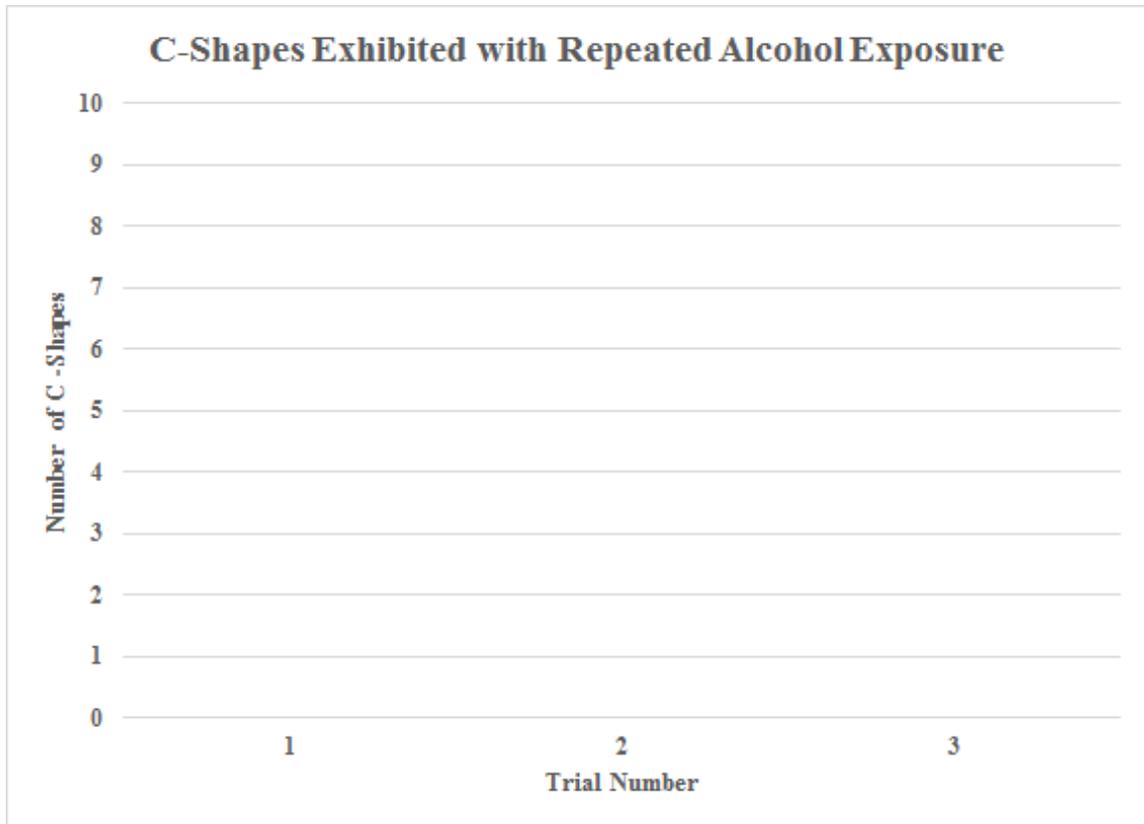
Trial	1	2	3
# of C-shapes			



ALCOHOL

1. Prepare one petri dish with a 0.1% solution of alcohol and one petri dish filled with spring water and place both on a plain white piece of paper. **You will want to test out the drug concentration under your own laboratory conditions. We recommend starting with 0.1%. You may need to adjust the concentration slightly down (e.g. 0.01%) if the planarians die with repeated exposure or slightly up (e.g. 1%) if the response is not robust enough.**
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the alcohol solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph below with your results

Trial	1	2	3
# of C-shapes			



Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

Which drugs cause a planarian to exhibit sensitization or tolerance over chronic use?

What might have been our **hypothesis**?

X drug(s) cause sensitization while X drug(s) cause tolerance.

What **methods** did we use to test our hypothesis?

We exposed planarians to the same drug repeatedly and measured the number of C-Shaped curves that each planarian exhibited in response to the chronic drug use.

What were our **results**?

Some planarians experienced sensitization while others experienced tolerance.

What **conclusions** can we draw from our results?

Chronic drug use can have two large effects on planarians (and on humans!). In some cases, chronic exposure of a drug will cause tolerance, and organisms respond less and less to the same concentration of drug. In other cases, chronic exposure can cause sensitization, and organisms will have increasingly strong responses to the same concentration of drug because the body has been primed to respond in a certain way.

Part 5: Discussion Questions

1. Looking at the graphs you filled in, which substance(s) showed sensitization? How can you tell?

The substances that showed sensitization will have increasing levels of C-Shaped curves with additional exposures.

2. Looking at the graphs you filled in, which substance(s) showed tolerance? How can you tell?

The substances that showed tolerance will have decreasing levels of C-Shaped curves with additional exposures.

3. In the case of tolerance, the planarian gets used to the substance concentration. To get the same response (number of C-shape movements) as you did in Trial 1, what could you do to the concentration of the substance?

You would need to increase the concentration of the substance

4. Do you think the planarian could have developed dependence? Why or why not?

Yes, because the planarian has been chronically exposed to a substance and now is reacting differently to the same concentrations.

5. How could you test whether or not the planarian has developed dependence?

To test if the planarian has developed dependence you could remove the planarian from the drug and see if it experiences signs of withdrawal.

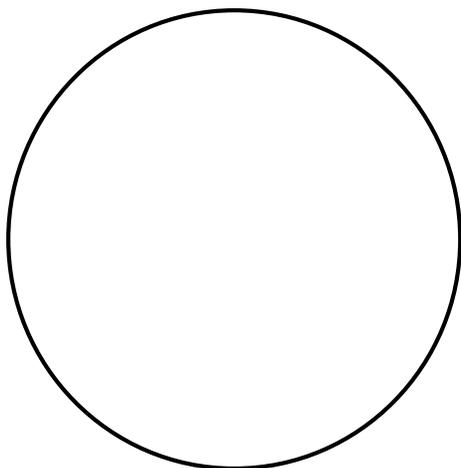
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Part 2: Observation

1. Examine the planarian under a microscope and draw what you see. List your observations below.



Part 3: Experiment

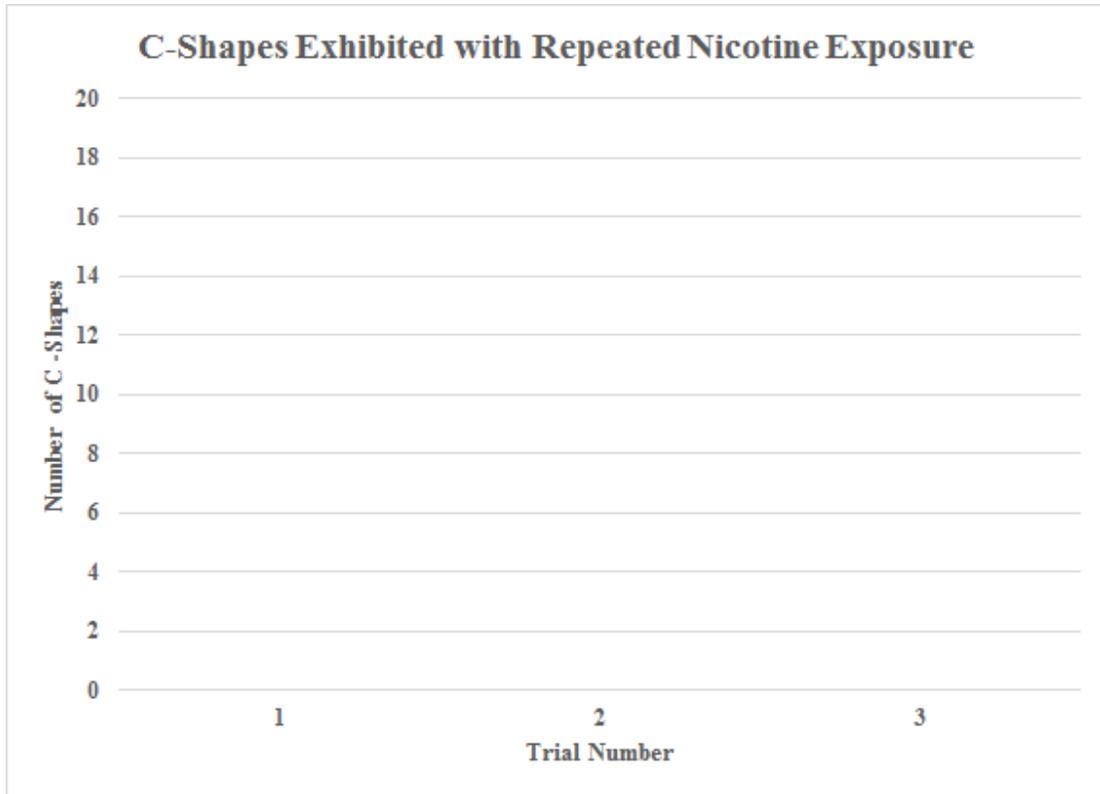
How is a planarian affected by repeated exposure to nicotine, caffeine, and alcohol?

Note: each group will be assigned one of the above substances to investigate

NICOTINE

1. Prepare one petri dish with a 1 mM solution of nicotine and one petri dish filled with spring water and place both on a plain white piece of paper.
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the nicotine solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph with your results below

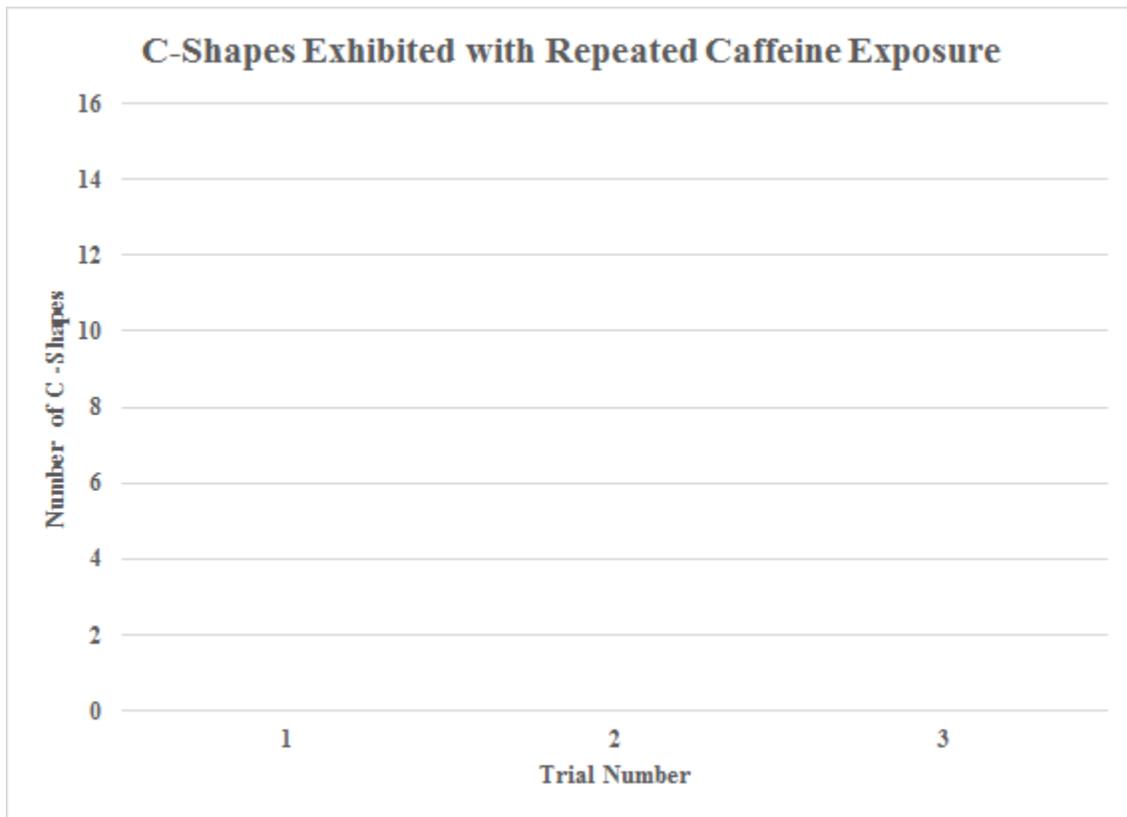
Trial	1	2	3
# of C-shapes			



CAFFEINE

1. Prepare one petri dish with a 1 mM solution of caffeine and one petri dish filled with spring water and place both on a plain white piece of paper.
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the caffeine solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph with your results below

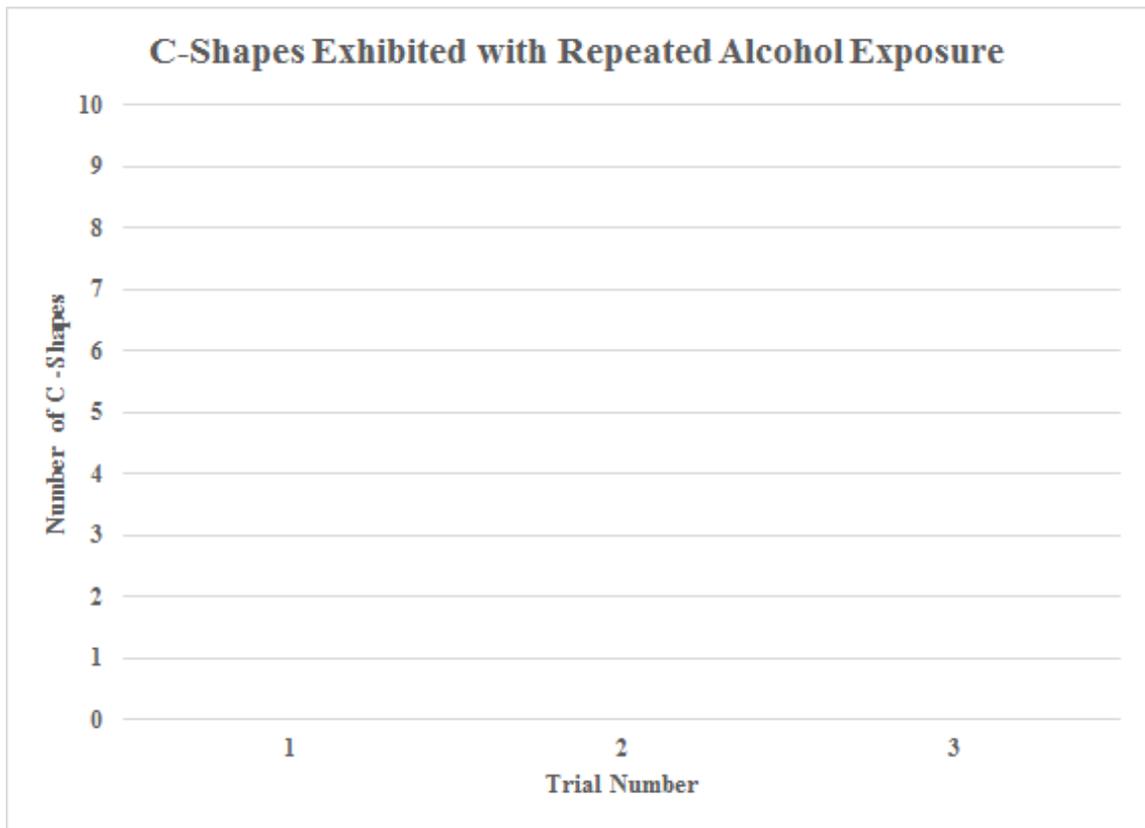
Trial	1	2	3
# of C-shapes			



ALCOHOL

1. Prepare one petri dish with a 0.1% solution of alcohol and one petri dish filled with spring water and place both on a plain white piece of paper.
2. Using a pipette, remove the planarian from its home jar
3. Using a pipette, place the planarian into the alcohol solution
4. For 2 minutes, count the C-shapes and record this number in the table below
5. Using a spatula, place the planarian into the spring water for 8 minutes
6. While you wait, exchange data from your first trial with the other groups
7. Repeat steps **3-6** two more times
8. Return the planarian to its home jar
9. Exchange information from your third trial with the other groups
10. Fill in the graph below with your results

Trial	1	2	3
# of C-shapes			



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What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. Looking at the graphs you filled in, which substance(s) showed sensitization? How can you tell?
2. Looking at the graphs you filled in, which substance(s) showed tolerance? How can you tell?
3. In the case of tolerance, the planarian gets used to the substance concentration. To get the same response (number of C-shape movements) as you did in Trial 1, what could you do to the concentration of the substance?
4. Do you think the planarian could have developed dependence? Why or why not?
5. How could you test whether or not the planarian has developed dependence?

Teacher's Guide: Acute Motility-- 55 minutes total

Goal: To demonstrate using planarians that drugs of abuse produce changes in motility. In rodents, low doses of stimulants (nicotine, caffeine, cocaine, amphetamines) produce an increase in horizontal or circular movement, which is defined as ambulation. Moderate doses produce more stereotypy (rearing, grooming, scratching, head twitches) with less ambulation. High doses produce almost exclusive stereotypy with very little ambulation. The same dose-profile is observed in planarians. If you use this lesson with Lesson 1 (Acute Stereotypy), you will note that increasing concentrations of a stimulant will produce more stereotypy and less motility.

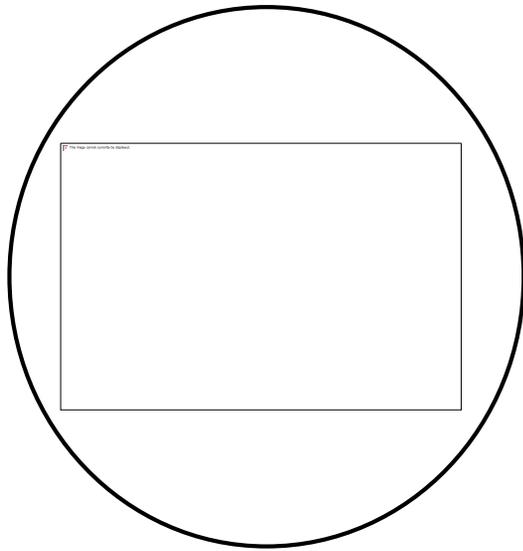
Part 1: Vocabulary--approximately 5 minutes

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Concentration	The amount of dissolved solute per unit volume of solution
Acute	A response of rapid onset and/or short duration
Stimulant	Something that will raise the level of activity in the body (especially in the nervous and cardiovascular systems) Examples: Nicotine and Caffeine
Depressant	Something that will decrease the level of activity in the body (especially in the nervous system) Example: Alcohol
Motility	Exhibiting or being capable of movement

Part 2: Observation--approximately 10 minutes

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope and list your observations.

Under observations students should note color, movement, size, shape, etc. It is recommended that the microscopes are set up for the students.



Part 3: Experiment--*approximately 20-25 minutes (including group sharing of results)*

How is a planarian affected by nicotine, caffeine, and alcohol? Note: each group will be assigned one of the above substances to investigate

Break the class into three groups and assign each group to one of the three conditions (nicotine, caffeine, or alcohol). This portion should take 25 minutes (5 concentrations x 5 minutes per concentration). Have the students share their results with other groups (e.g. via white board) before moving onto the discussion questions.

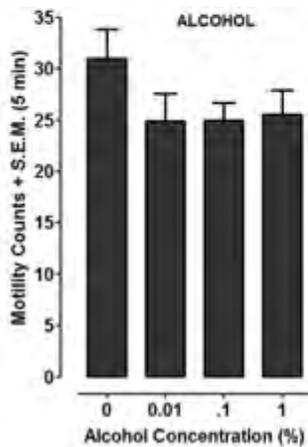
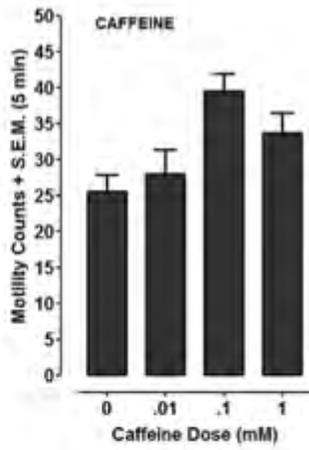
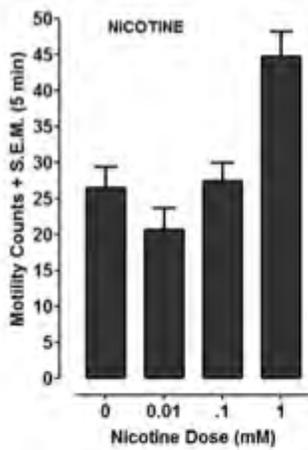
NICOTINE

1. Label 2 petri dishes, one with "Spring Water" and a second with "0.1 mM Nicotine"
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes? **note: if you are counting lines from the planarian's head, make sure all students are counting from the planarian's head-- keep the scale consistent across your class!*
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of nicotine.
5. Gently use a spatula to move the planarian from the Spring Water to the Nicotine.
6. How many lines does the planarian cross in 3 minutes?
7. When finished, place the planarian back in the Spring Water dish. *You can manipulate the time between trials to best fit the timing needs of your classroom.*
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Nicotine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. **Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose. It is recommended that you pilot the experiment the day before planning the activity with students to test the concentrations under your own laboratory conditions.**

Solution	0 mM (spring water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	26	30	45		
Trial 2					
Average					

****Note: these are the values obtained with our own experimental procedure, they may vary slightly from classroom to classroom. It is recommended that you test these on your own first!**
Fill in the graph with the average from your results below.



CAFFEINE

1. Label 2 petri dishes, one with “Spring Water” and a second with “0.1 mM Caffeine”
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes? **note: if you are counting lines from the planarian’s head, make sure all students are counting from the planarian’s head-- keep the scale consistent across your class!*
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of Caffeine.
5. Gently use a spatula to move the planarian from the Spring Water to the Caffeine.
6. How many lines does the planarian cross in 3 minutes?
7. When finished, place the planarian back in the Spring Water dish. *You can manipulate the time between trials to best fit the timing needs of your classroom.*
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Caffeine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. *Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose.*

Solution	0 mM (Spring Water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	25	40	35		
Trial 2					
Average					

***Again, these values are results obtained for our own experimental procedure and will vary!*

Fill in the graph with the average from your results below.
Example graphs for each substance can be found above

ALCOHOL

1. Label 2 petri dishes, one with “Spring Water” and a second with “0.1 mM Alcohol”
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes? **note: if you are counting lines from the planarian’s head, make sure all students are counting from the planarian’s head-- keep the scale consistent across your class!*
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of Alcohol.
5. Gently use a spatula to move the planarian from the Spring Water to the Alcohol.
6. How many lines did the planarian cross in 3 minutes?

7. When finished, place the planarian back in the Spring Water dish. **You can manipulate the time between trials to best fit the timing needs of your classroom.**
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Alcohol concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below. **Note: with a concentration of 10 (and maybe even 5) dose of drug may become so high that it is toxic to the planarian. If you do not want this to occur in your classroom just alter the experiment to eliminate that high dose.**

Solution	0 mM (Spring Water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1	30	25	25		
Trial 2					
Average					

****Note, results may vary**

Fill in the graph with the average from your results below.
Example graphs for each substance can be found above

Part 4: Scientific Method Recap--approximately 10 minutes

What **question** were we trying to answer with this experiment?

What are the differences in motility when we are using a stimulant versus a depressant?

What might have been our **hypothesis**?

Stimulants will increase motility, while depressants will decrease motility.

Note: a valid hypothesis might also predict the reverse, or might predict no effects on motility at all.

What **methods** did we use to test our hypothesis?

We placed the planarians in increasing concentrations of 2 different stimulants (Caffeine and Nicotine) and 1 depressant (Alcohol) and counted the number of lines each planarian crossed in 5 minutes.

What were our **results**?

Both Nicotine and Caffeine were associated with increased motility, while Alcohol is associated with decreased motility

What **conclusions** can we draw from our results?

Stimulants increase motility and depressants decrease motility

Part 5: Discussion Questions--*approximately 10 minutes*

1. What patterns do you notice as the concentrations increase?
In the stimulants (caffeine and nicotine) as the concentrations increased, generally so did motility. In the depressant (alcohol), as the concentration increased, motility decreased.
2. Is there a relationship between drug concentration and planarian motility?
Yes. Increased concentrations of drug have an increased effect on planarian movement.
3. Is there a difference in motility when you place a planarian in a stimulant (Caffeine or Nicotine) versus a depressant (Alcohol)? If so, what?
In stimulants, planarians have increased motility. In depressants, planarians have decreased motility.
4. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how these substances (nicotine, caffeine, alcohol, etc.) affect our own bodies?
If these substances are having physical effects on planarians, then they probably will have physical effects on humans as well. Also, in general, stimulants will tend to increase our activity, whereas depressants will decrease our activity.

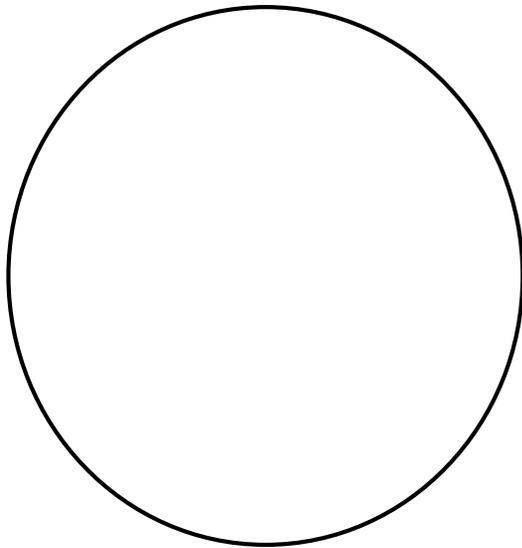
Acute Motility

Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Concentration	The amount of dissolved solute per unit volume of solution
Acute	A response of rapid onset and/or short duration
Stimulant	Something that will raise the level of activity in the body (especially in the nervous and cardiovascular systems) Examples: Nicotine and Caffeine
Depressant	Something that will decrease the level of activity in the body (especially in the nervous system) Example: Alcohol
Motility	Exhibiting or being capable of movement

Part 2: Observation

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope and list your observations.



Part 3: Experiment

How is a planarian affected by nicotine, caffeine, and alcohol? Note: each group will be assigned one of the above substances to investigate

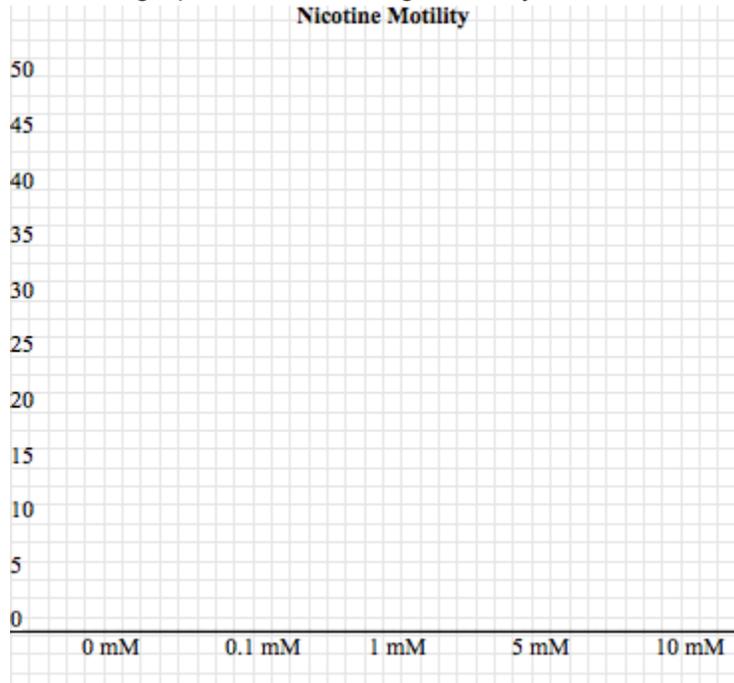
NICOTINE

1. Label 2 petri dishes, one with “Spring Water” and a second with “0.1 mM Nicotine”
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes?
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of nicotine.
5. Gently use a spatula to move the planarian from the Spring Water to the Nicotine.
6. How many lines does the planarian cross in 3 minutes?
7. When finished, place the planarian back in the Spring Water dish.
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Nicotine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

Solution	0 mM (spring water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1					
Trial 2					
Average					

Fill in the graph with the averages from your results below.



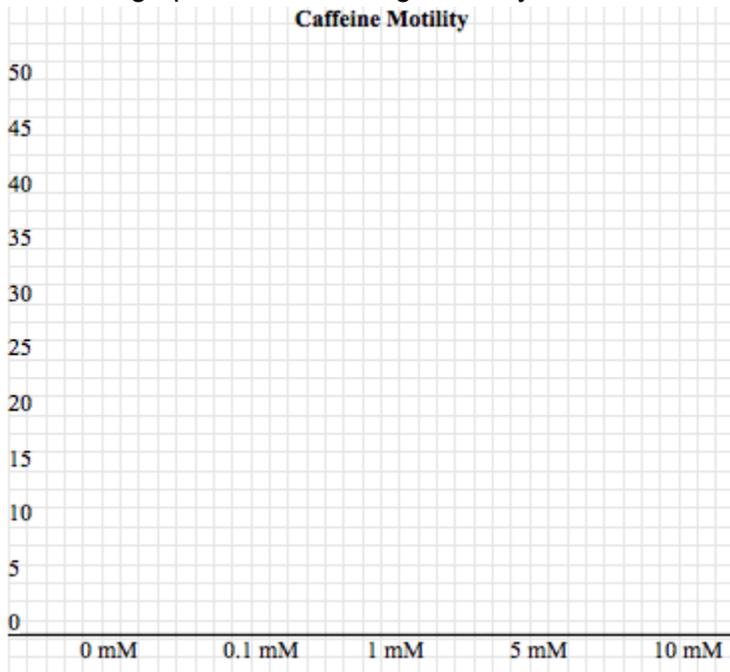
CAFFEINE

1. Label 2 petri dishes, one with “Spring Water” and a second with “0.1 mM Caffeine”
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes?
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of Caffeine.
5. Gently use a spatula to move the planarian from the Spring Water to the Caffeine.
6. How many lines does the planarian cross in 3 minutes?
7. When finished, place the planarian back in the Spring Water dish
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Caffeine concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

Solution	0 mM (Spring Water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1					
Trial 2					
Average					

Fill in the graph with the averages from your results below.



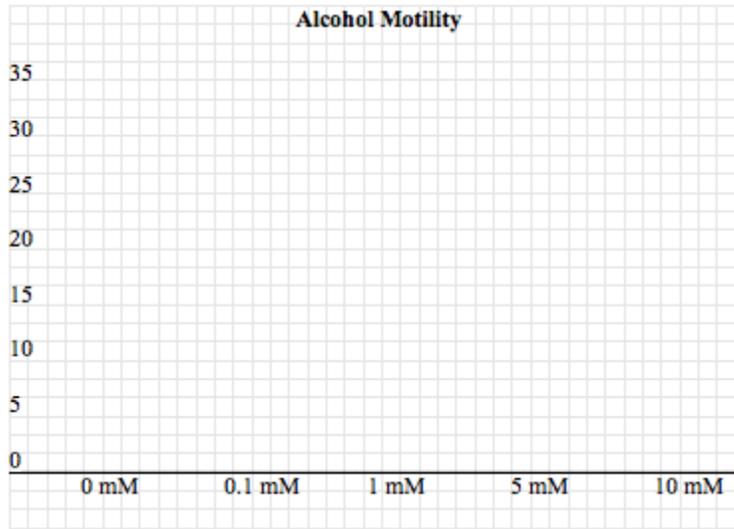
ALCOHOL

1. Label 2 petri dishes, one with “Spring Water” and a second with “0.1 mM Alcohol”
2. On a sheet of graph paper, place your planarian on a petri dish with Spring Water. How many lines does the planarian cross in 3 minutes?
3. Record your observations in the table below.
4. On a sheet of graph paper place a second petri dish with a 0.1 mM solution of Alcohol.
5. Gently use a spatula to move the planarian from the Spring Water to the Alcohol.
6. How many lines does the planarian cross in 3 minutes?
7. When finished, place the planarian back in the Spring Water dish.
8. Repeat steps 1-7 with a different planarian.

Repeat the above with Alcohol concentrations of 1 mM, 5 mM and 10 mM. Record your findings in the table below.

Solution	0 mM (Spring Water)	0.1 mM	1.0 mM	5.0 mM	10 mM
Trial 1					
Trial 2					
Average					

Fill in the graph with the averages from your results below.



Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. What patterns do you notice as the concentrations increase?

2. Is there a relationship between drug concentration and planarian motility?

3. Is there a difference in motility when you place a planarian in a stimulant (Caffeine or Nicotine) versus a depressant (Alcohol)? If so, what?

4. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how these substances (nicotine, caffeine, alcohol, etc.) affect our own bodies?

Environmental Experiment (Light versus Dark to Study Anxiety)-**about 50 minutes**

Goal: Anxiety disorders are the most common mental illness in the United States and affects about 18% of the US population over the age of 18. Anxiety is an emotion characterized by an unpleasant state of inner turmoil. Anxiogenic is a term that means “anxiety-producing” and anxiolytic is a term that means “anxiety-relieving”. The most common preclinical models that are used to study anxiety in rats and mice are the light/dark box and the elevated plus maze. Animals that are more anxious tend to spend a greater amount of time in a dark or closed compartment. In contrast, animals that are less anxious tend to spend more time in light or open spaces. Planarians also tend to spend a greater amount of time in the dark when given a choice between a light and dark compartment. And, the preference for the dark is believed to be an indicator of enhanced anxiety.

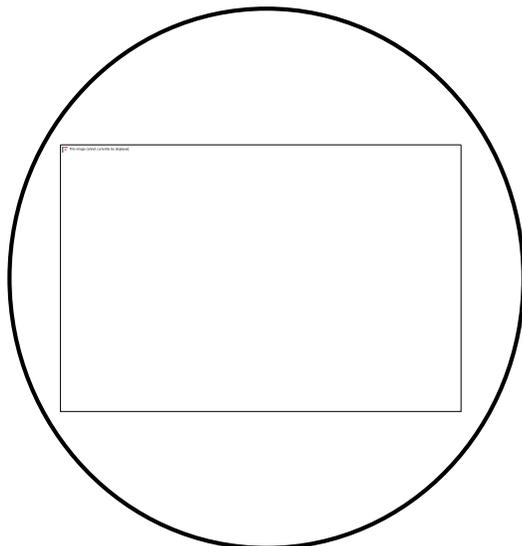
Part 1: Vocabulary-**5 minutes**

Planarian	a type of flatworm and the subject of this experiment
Eyespots	primitive eyes that can distinguish light from dark
Predator	an animal that kills and eats other animals (e.g. a wolf)
Prey	an animal that is killed and eaten by other animals (e.g. a rabbit)
Instinct	an inborn pattern of behavior
Anxiety	a feeling of worry, nervousness, or unease

Part 2: Observation and Experiment-**25 minutes**

1. Examine the planarian under a microscope and list your observations below. Draw a picture of what you see.

Size, shape, color, eye spots, etc.



2. Place a petri dish filled with spring water on a white piece of paper.
3. Cover half of the dish with black paper.
4. For 5 minutes, record where the planarian spends its time.
5. Repeat steps 2-4 with a second planarian.

	Planarian 1: Time Spent (minutes)	Planarian 2: Time Spent (minutes)
Light Side	~2 minutes	
Dark Side	~8 minutes	

Part 3: Scientific Method Recap-10 minutes

What **question** were we trying to answer with this experiment?

Does the planarian prefer to spend more time in the light or dark?

What might have been our **hypothesis**?

Either: The planarian prefers to spend time in the light because _____

The planarian prefers to spend time in the dark because _____

What **methods** did we use to test our hypothesis?

We covered the petri dish and recorded how long the planarian spent under the black paper and how long it spent in the light.

What were our **results**?

The planarian spent most (~80%) of its time under the dark paper.

What **conclusions** can we draw from our results?

Planarians prefer to spend more time in the dark than in the light.

Part 4: Discussion Questions-10 minutes

1. On which side did the planarian spend more of its time?

The dark side.

2. Do any features you noted make the planarian seem like a predator? If so, which ones?

There shouldn't be any, but kids can be creative.

3. Do any features you noted make the planarian seem like prey? If so, which ones?

Soft body, lack of visible defenses, etc.

4. Based on your observations, would you think that planarians in the wild spend their most of their time in sunny parts of streams or in shady parts next to rocks?

Shady parts next to rocks

5. Why do you think the animal might experience anxiety when spending time away from there?

Planarians are easy targets for predators/ they are more safe by the rocks/etc.

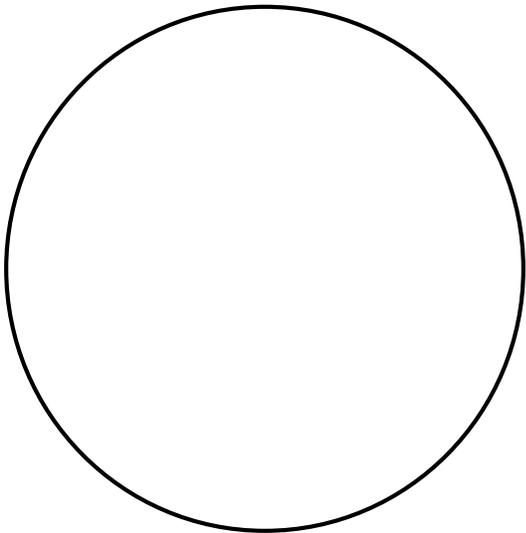
Environmental Experiment (Light versus Dark)

Part 1: Vocabulary

Planarian	a type of flatworm and the subject of this experiment
Eyespots	primitive eyes that can distinguish light from dark
Predator	an animal that kills and eats other animals (e.g. a wolf)
Prey	an animal that is killed and eaten by other animals (e.g. a rabbit)
Instinct	an inborn pattern of behavior
Anxiety	a feeling of worry, nervousness, or unease

Part 2: Observation and Experiment

1. Examine the planarian under a microscope and list your observations below. Draw a picture of what you see.



2. Place a petri dish filled with spring water on a white piece of paper
3. Cover half the dish with black paper.
4. For 5 minutes, record where the planarian spends its time.
5. Repeat steps 2-4 with a second planarian.

	Planarian 1: Time Spent (minutes)	Planarian 2: Time Spent (minutes)
Light Side		
Dark Side		

Part 3: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 4: Discussion Questions

1. On which side did the planarian spend more of its time?
2. Do any features you noted make the planarian seem like a predator? If so, which ones?

3. Do any features you noted make the planarian seem like prey? If so, which ones?

4. Based on your observations, would you think that planarians in the wild spend their most of their time in sunny parts of streams or next to rocks?

5. Why do you think the animal might experience anxiety when spending time away from there?

Drug Withdrawal-induced Anxiety – **Would probably want to conduct this experiment over 2 days**

Goal: Drug abusers that discontinue chronic consumption of addictive substances display a negative motivational state including depression and anxiety that increases vulnerability to relapse. This anxiety and depression during withdrawal are symptoms of psychological dependence that intensify craving and cause drug abusers to relapse to drug seeking and taking. This is called the ‘dark side’ of addiction. Rats or mice withdrawn from chronic regimens of an addictive substance spend more time in dark (e.g. light/dark box) or closed (e.g. elevated plus maze assay) compartments. Classical benzodiazepine anxiolytics are active in these mammalian models where they reduce the anxiogenic-type response during alcohol or cocaine withdrawal and are the front-line therapy for treating anxiety due to acute alcohol withdrawal syndrome. We have developed a model using planarians that can mimic the rat and mouse models and can be used as a pre-mammalian screen for anxiety. Essentially, planarians exposed to an addictive substance and then withdrawn from the substance and placed into a split dish (light/dark) containing water will spend a greater amount of time in the dark compartment, indicating increased anxiety during drug withdrawal.

Part 1: Vocabulary--5-10 minutes

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Control Group	A group or individual (in this case, planarian), used as a reference for comparison when doing an experiment or survey. This group does not receive drug or treatment.
Experimental Group	A group or individual that the researchers manipulate to test a hypothesis.
Withdrawal	Physical and physiological symptoms that happen when an individual stops taking an addictive drug. Symptoms can include: anxiety, sweating, goosebumps, vomiting, muscle pain and insomnia
Physical Dependence	A state resulting from chronic use of a drug where negative physical symptoms of withdrawal result from abrupt discontinuation or dosage reduction.

Part 2: Observations--approximately 10 minutes

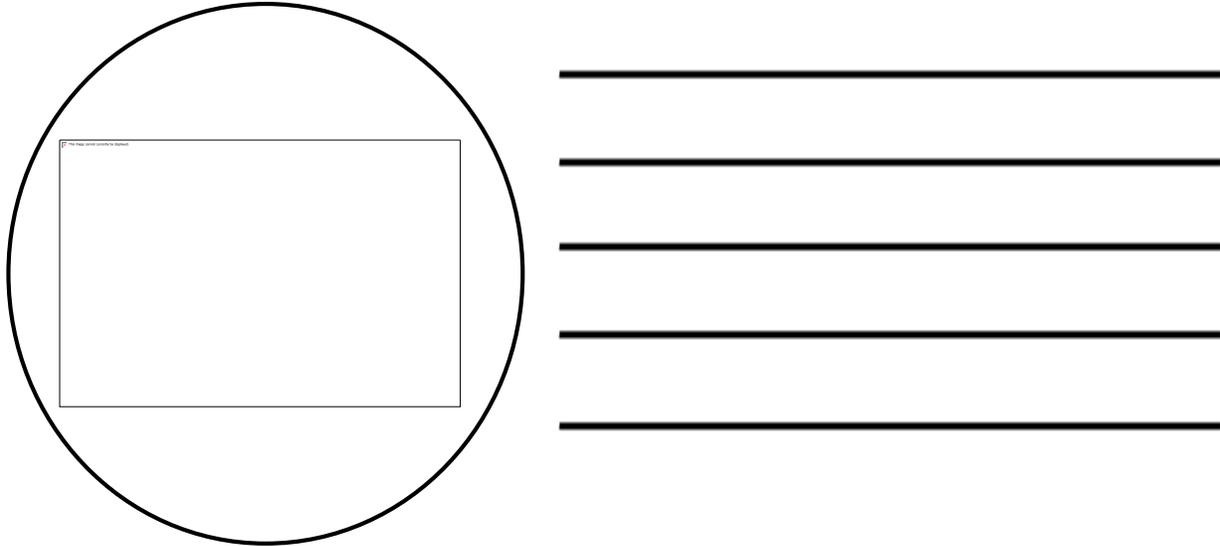
1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.

2. Draw a picture in the circle below of what you saw under the microscope and list your observations.

Under observations students should note color, movement, size, shape, etc.

It is recommended that the microscopes

are set up for the students.



Part 3: Experiment

Experiment – approximately 40 min (20 min for each planarian) (Note that you may want to conduct this experiment over 2 days. If time is a concern, you may want to shorten the exposure interval from 10 to 5 min. However, we suggest that you try this experiment as it is a good hands-on approach for teaching the anxiety that can result following discontinuation of chronic drug exposure or consumption.)

1. Label a petri dish with “spring water” and fill it with spring water. Label a second petri dish with “drug” and fill it with 0.1 mM (nicotine, caffeine) or 0.1% (alcohol, sucrose).
2. Place a planarian in the petri dish labeled “spring water” for 10 minutes.
3. Take a new petri dish and cover half of the top with black construction paper. Both the bottom and the top of the petri dish should be covered with the black construction paper to create two compartments of equal size (one light compartment and one dark compartment). Fill the petri dish with spring water.
4. Remove the planarian from the dish labeled “spring water” and gently place it at the midline of the petri dish with the black construction paper to allow it free access to roam both the light and dark compartments.
5. Observe and record the amount of time each planarian spends on the light versus the dark side of the plate over the course of the next 5 minutes. Record your observations in the table below.
6. Place a planarian in the petri dish labeled “drug” for 10 minutes.

7. Remove the planarian from the dish labeled “drug” and gently place it at the midline of the petri dish with the black construction paper to allow it free access to roam both the light and dark compartments.
8. Observe and record the amount of time each planarian spends on the light versus the dark side of the plate over the course of the next 5 minutes. Record your observations in the table below.
9. Repeat steps 1-8 with additional planarians, for a total of 2 planarians in the drug group and 2 in the spring water group. **This step is important in case a student breaks a planarian, and also to increase the sample size being used in this experiment.**

Planarian Exposure	Time in the Light	Time in the Dark
Water Group 1	some time	more time
Water Group 2		
Drug Group 1	very little time	most time
Drug Group 2		

****Note:** Experimental times will vary slightly, you might want to try this experiment out first to obtain your own data. You might also try soaking the planarians in the spring water and drug solutions overnight and then running the light/dark experiment the following day. In this case, for the longer exposure interval, a lower concentration of drug is recommended.

Part 4: Scientific Method Recap-approximately 10 minutes

What **question** were we trying to answer with this experiment?

Which group of planarians had increased anxiety (indicative of withdrawal?)

What might have been our **hypothesis**?

A valid hypothesis might predict that the drug exposed group would have increased anxiety, that the non-drug exposed group would have increased anxiety, or that there is no difference between the anxiety levels of each group.

What **methods** did we use to test our hypothesis?

We exposed an experimental group to a drug for 24 hours, and then removed it from the drug environment (prompting it to go through withdrawal). We then measured its anxiety levels by looking at the amount of time it spent on the light versus dark side of a petri dish.

What were our **results**?

The drug exposed group spent more time on the dark side of the plate.

What **conclusions** can we draw from our results?

The drug exposed group had increased anxiety levels following removal of the drug.

Part 5: Discussion Questions--approximately 15 minutes

1. In the above experiment, which group of planarians were functioning as our control group? Which were our experimental group? How do you know?

The control group was the group exposed to water overnight. The experimental group was the group exposed to the drug overnight. We know which is which because we are manipulating the environment of the drug exposed group, and leaving the environment of the control group unchanged.
2. In general planarians prefer to spend time in the dark. Why do you think that might be the case?

Planarians can hide from predators more easily in the dark.
3. Which group spent more time in the dark?

The drug exposed group spent more time in the dark.
4. One of the hallmarks of withdrawal is increased anxiety levels. Which group of planarians would you predict had increased anxiety? How can you tell?

The drug exposed group had increased anxiety levels . You can tell because they chose to spend more time in the dark.
5. What part of our experiment might mimic withdrawal?

Exposing the planarians to a drug and then removing them from that environment mimics withdrawal.
6. Why would going through withdrawal change the amount of time a planarian spends in the light versus the dark?

Withdrawal from drugs often includes increased anxiety levels. Therefore when you remove the planarian from the drug it will have increased anxiety and choose to spend more time in the dark to try to ease their anxiety.
7. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how drugs (and withdrawal from drugs) affect our own bodies?

This suggests that humans also might have increased anxiety levels when they taking drugs.

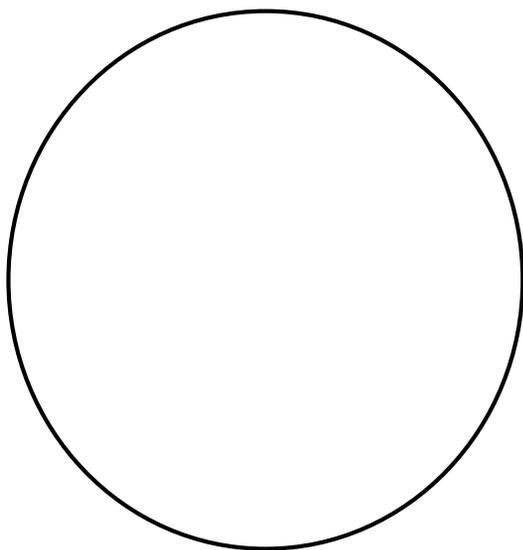
Drug Withdrawal-induced Anxiety

Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Control Group	A group or individual (in this case, planarian), used as a reference for comparison when doing an experiment or survey. This group does not receive drug or treatment.
Experimental Group	A group or individual that the researchers manipulate to test a hypothesis.
Withdrawal	Physical and physiological symptoms that happen when an individual stops taking an addictive drug. Symptoms can include: anxiety, sweating, goosebumps, vomiting, muscle pain and insomnia
Physical Dependence	A state resulting from chronic use of a drug where negative physical symptoms of withdrawal result from abrupt discontinuation or dosage reduction.

Part 2: Observations

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope and list your observations.



Part 3: Experiment

Experiment -

1. Label a petri dish with “spring water” and fill it with spring water. Label a second petri dish with “drug” and fill it with 0.1 mM (nicotine, caffeine) or 0.1% (alcohol, sucrose).
2. Place a planarian in the petri dish labeled “spring water” for 10 minutes.
3. Take a new petri dish and cover half of the top with black construction paper. Both the bottom and the top of the petri dish should be covered with the black construction paper to create two compartments of equal size (one light compartment and one dark compartment). Fill the petri dish with spring water.
4. Remove the planarian from the dish labeled “spring water” and gently place it at the midline of the petri dish with the black construction paper to allow it free access to roam both the light and dark compartments.
5. Observe and record the amount of time each planarian spends on the light versus the dark side of the plate over the course of the next 5 minutes. Record your observations in the table below.
6. Place a planarian in the petri dish labeled “drug” for 10 minutes.
7. Remove the planarian from the dish labeled “drug” and gently place it at the midline of the petri dish with the black construction paper to allow it free access to roam both the light and dark compartments.
8. Observe and record the amount of time each planarian spends on the light versus the dark side of the plate over the course of the next 5 minutes. Record your observations in the table below.
9. Repeat steps 1-8 with additional planarians, for a total of 2 planarians in the drug group and 2 in the spring water group.

Planarian Exposure	Time in the Light	Time in the Dark
Water Group 1		
Water Group 2		
Drug Group 1		
Drug Group 2		

Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. In the above experiment, which group of planarians were functioning as our control group? Which were our experimental group? How do you know?
2. In general planarians prefer to spend time in the dark. Why do you think that might be the case?
3. Which group spent more time in the dark?
4. One of the hallmarks of withdrawal is increased anxiety levels. Which group of planarians would you predict had increased anxiety? How can you tell?
5. What part of our experiment might mimic withdrawal?
6. Why would going through withdrawal change the amount of time a planarian spends in the light versus the dark?

7. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how drugs (and withdrawal from drugs) affect our own bodies?

Depression

Goal: Drug abusers that discontinue chronic consumption of addictive substances display a negative motivational state including depression and anxiety that increases vulnerability to relapse. This anxiety and depression during withdrawal are symptoms of psychological dependence that intensify craving and cause drug abusers to relapse to drug seeking and taking. This is called the 'dark side' of addiction. Rats or mice withdrawn from chronic regimens of an addictive substance tend to display depressed behavioral responses. For example, the most common rodent model for examining depression-like behavior is the forced swim test (FST). In short, a rat withdrawn from a chronic regimen of an addictive substance is placed into a container of water. The rat undergoing drug withdrawal will tend to become 'immobile' faster than control rats. The increased immobility, and the increased onset of the immobility, is believed to model depression in that the rat gives up and stops trying to escape. We have developed a model using planarians that can mimic the rat and mouse models and can be used as a pre-mammalian screen for depression. Essentially, planarians exposed to an addictive substance and then withdrawn from the substance and placed into a dish containing water (with graph paper underneath) will display decreased motility relative to water controls. This is believed to represent a depressed behavioral response caused by drug withdrawal.

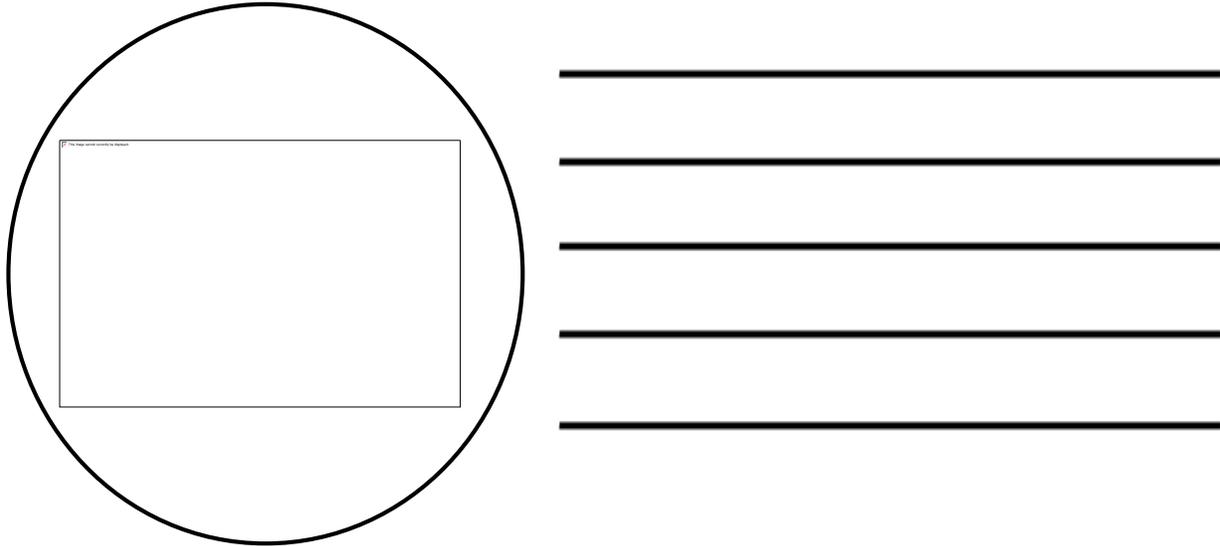
Part 1: Vocabulary--5-10 minutes

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Control Group	A group or individual (in this case, planarian), used as a reference for comparison when doing an experiment or survey. This group does not receive drug or treatment.
Experimental Group	A group or individual that the researchers manipulate to test a hypothesis.
Withdrawal	Physical and physiological symptoms that happen when an individual stops taking an addictive drug. Symptoms can include: anxiety, sweating, goosebumps, vomiting, muscle pain and insomnia
Physical Dependence	A state resulting from chronic use of a drug where negative physical symptoms of withdrawal result from abrupt discontinuation or dosage reduction.

Part 2: Observations--approximately 10 minutes

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope and list your observations.

Under observations students should note color, movement, size, shape, etc.
It is recommended that the microscopes are set up for the students.



Part 3: Experiment

Experiment – approximately 40 min (10 min for each planarian). Students will need 4 total petri dishes for the experiment. If students are paired in groups of 2, it may be possible to have them run more planarians by having them both work on a different worm simultaneously. It is recommended that you pool the results of all the individual student groups at the end of the experiment to obtain an overall class average or mean. This is also an ideal experiment to test different concentrations of the same drug or to compare effects of different classes of drugs. For example, if you wanted to extend the experiments to a second day, you could have the students test various concentrations of a single drug (e.g. sucrose – 0.01, 0.1 and 1%). This would teach students how concentration impacts response. You could also have them compare different classes of substances (nicotine and caffeine are stimulants; alcohol is a depressant; and sucrose is a natural reinforcer).

1. Label a petri dish with “spring water” and fill it with spring water.
2. Place a single planarian in this dish and leave it for 5 minutes. During this 5-minute-soak, label another petri dish with “spring water/withdrawal” and fill this dish with spring water. Place a piece of graph paper underneath the “spring water/withdrawal” petri dish.

- Remove the planarian from the dish labeled “spring water” and gently place it in the “spring water/withdrawal” dish. Over the course of the next 5 minutes, count the number of times the head of planarian crosses or recrosses a single interval on the graph paper. Record your observations below. **It is recommended that you demonstrate how to count the grid crossings prior to having students conduct the experiment. Also stress to the students to be consistent they count the crossings. We normally only count forward movement meaning that the head of the planarian crosses (or recrosses) an interval.**
- Label a second petri dish with “drug” and fill it with 0.1 mM (nicotine, caffeine) or 0.1% (alcohol, sucrose).
- Place a single planarian in this dish and leave it for 5 minutes. During this 5-minute-soak, label another petri dish with “spring water/withdrawal” and fill this dish with spring water. Place a piece of graph paper underneath the “spring water/withdrawal” petri dish.
- Remove the planarian from the dish labeled “drug” and gently place it in the “spring water/withdrawal” dish. Over the course of the next 5 minutes, count the number of times the head of planarian crosses or recrosses a single interval on the graph paper. Record your observations below.
- Repeat steps 1-6 and record your observations below.

Planarian Exposure	Number of Lines Crossed
Water Group 1	some lines
Water Group 2	
Drug Withdrawal Group 1	fewer lines
Drug Withdrawal Group 2	

****Note: Experimental times will vary slightly, you might want to try this experiment out first to obtain your own data.**

Part 4: Scientific Method Recap-approximately 10 minutes

What **question** were we trying to answer with this experiment?

Which group of planarians had increased depression (indicative of withdrawal?)

What might have been our **hypothesis**?

A valid hypothesis might predict that the drug exposed group would have increased depression, that the non-drug exposed group would have increased depression, or that there is no difference between the depression levels of each group.

What **methods** did we use to test our hypothesis?

We exposed an experimental group to a drug for 24 hours, and then removed it from the drug environment (prompting it to go through withdrawal). We then measured its depression levels by measuring the planarian's motility following removal from the drug environment.

What were our **results**?

The drug exposed group had decreased motility.

What **conclusions** can we draw from our results?

The drug exposed group had increased depression levels following removal of the drug.

Part 5: Discussion Questions--approximately 15 minutes

1. In the above experiment, which group of planarians were functioning as our control group? Which were our experimental group? How do you know?

The control group was the group exposed to water overnight. The experimental group was the group exposed to the drug overnight. We know which is which because we are manipulating the environment of the drug exposed group, and leaving the environment of the control group unchanged.

2. Which group had decreased motility?

The drug exposed group had decreased motility.

3. One of the hallmarks of withdrawal is increased depression levels. Which group of planarians would you predict had increased depression? How can you tell?

The drug exposed group had increased depression levels. You can tell because they had decreased motility.

4. What part of our experiment might mimic withdrawal?

Exposing the planarians to a drug and then removing them from that environment mimics withdrawal.

5. Why would going through withdrawal change the amount of time a planarian's motility?

Withdrawal from drugs often includes increased depression levels. Therefore when you remove the planarian from the drug it will have increased depression and experience lethargy, moving less.

6. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how drugs (and withdrawal from drugs) affect our own bodies?

This suggests that humans also might have increased depression levels when they taking drugs.

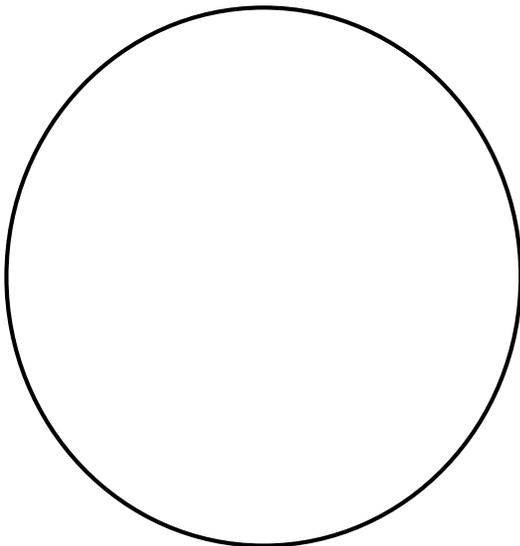
Depression

Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Control Group	A group or individual (in this case, planarian), used as a reference for comparison when doing an experiment or survey. This group does not receive drug or treatment.
Experimental Group	A group or individual that the researchers manipulate to test a hypothesis.
Withdrawal	Physical and physiological symptoms that happen when an individual stops taking an addictive drug. Symptoms can include: anxiety, sweating, goosebumps, vomiting, muscle pain and insomnia
Physical Dependence	A state resulting from chronic use of a drug where negative physical symptoms of withdrawal result from abrupt discontinuation or dosage reduction.

Part 2: Observations

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope and list your observations.



Part 3: Experiment

Experiment -

1. Label a petri dish with “spring water” and fill it with spring water.
2. Place a single planarian in this dish and leave it for 5 minutes. During this 5-minute-soak, label another petri dish with “spring water/withdrawal” and fill this dish with spring water. Place a piece of graph paper underneath the “spring water/withdrawal” petri dish.
3. Remove the planarian from the dish labeled “spring water” and gently place it in the “spring water/withdrawal” dish. Over the course of the next 5 minutes, count the number of times the head of planarian crosses or recrosses a single interval on the graph paper. Record your observations below.
4. Label a second petri dish with “drug” and fill it with 0.1 mM (nicotine, caffeine) or 0.1% (alcohol, sucrose).
5. Place a single planarian in this dish and leave it for 5 minutes. During this 5-minute-soak, label another petri dish with “spring water/withdrawal” and fill this dish with spring water. Place a piece of graph paper underneath the “spring water/withdrawal” petri dish.
6. Remove the planarian from the dish labeled “drug” and gently place it in the “spring water/withdrawal” dish. Over the course of the next 5 minutes, count the number of times the head of planarian crosses or recrosses a single interval on the graph paper. Record your observations below.
7. Repeat steps 1-6 and record your observations below.

Planarian Exposure	Number of Lines Crossed
Water Group 1	
Water Group 2	
Drug Withdrawal Group 1	
Drug Withdrawal Group 2	

Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. In the above experiment, which group of planarians were functioning as our control group? Which were our experimental group? How do you know?
2. Which group had decreased motility?
3. One of the hallmarks of withdrawal is increased depression levels. Which group of planarians would you predict had increased depression? How can you tell?
4. What part of our experiment might mimic withdrawal?
5. Why would going through withdrawal change the amount of time a planarian's motility?
6. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how drugs (and withdrawal from drugs) affect our own bodies?

Rewarding Effects (Conditioned Place Preference)-- approximately 90 minutes total

Goal: Drug abusers tend to associate elements of their environment with the rewarding and reinforcing effects of an addictive substance. For example, an alcoholic tends to frequent the same bars to drink and often drinks with the same group of friends. In this case, the alcoholic begins to associate the rewarding effect of the alcohol with the bar and his friends. The bar, and friends, serve as environmental cues that promote the alcoholic to seek and consume a drink. The drink itself is the unconditioned response whereas the bar and friends are the conditioned response. That is, being in the bar or in the presence of these friends makes it more likely the alcoholic will drink. Animal models have been developed to study this behavior and these models are called conditioned place preference. The model involves three phases. The first phase is a pre-test in which an animal is placed in an apparatus containing two compartments with different environmental cues. For example, in rat studies, one side may have a smooth surface and the opposing side may have a ruffled surface. The rat is placed in the middle of the chamber and given free access to roam both sides of the chamber. The rat will normally spend a greater amount of time on one side versus the other. The side on which the rat spends the least amount of time is designated as the least-preferred side. The second phase is the conditioning phase in which the rat is injected for 5-10 days with an addictive substance and confined to the least-preferred compartment. During this time, the rat is also injected with saline and confined to the more preferred compartment. The third phase is the post-test in which the pre-test is essentially repeated. That is, the rat is placed in the middle of the chamber and given access to roam both compartments. The rat, following conditioning, will now tend to spend a greater amount of time in the original least-preferred compartment. This 'preference shift' is thought to indicate that the rat is willing to explore the least-preferred environment to seek the drug or that the addictive substance has impaired the rat to an extent that it is engaging in risk-taking behaviors due to less than normal anxiety. We have developed a similar model using planarians that capitalizes upon the flatworms inherent tendency to spend more time in the dark.

Part 1: Vocabulary--5-10 minutes

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Preferred Environment	The environment an organism chooses to be in when given a choice
Non-preferred Environment	The environment an organism avoids when given a choice
Stimulus	Something that causes a change or reaction

Conditioning	A form of learning involving the formation, strengthening, or weakening of an association between a stimulus and a response
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Part 2: Pre-Test--approximately 10 minutes

1. Prepare a petri dish that is half covered by black construction paper and fill it with spring water.
2. Gently place a planarian into the petri dish, and record how much time it spends on the light versus the dark side of the plate over the course of 5 minutes. Record your observations below using seconds as your unit.

Time on the Light Side (seconds)	normally this side with be less time
Time on the Dark Side (seconds)	normally this side will be more time

3. Which side of the plate did the planarian spend more time on? dark,
normally_____ This is the planarian's **preferred environment**.
4. Which side of the plate did the planarian spend less time on? light,
normally_____ This is the planarian's **non-preferred environment**.

If you have a planarian whose preferred environment is the DARK, continue to part 3: Conditioning. However if your planarian prefers the light, please repeat part 2 with a different planarian. For the purposes of consistency in the following parts of the experiment, we need all conditioned planarians to be conditioned in the light, so please only have your students use planarians that prefer the dark (This should be the vast majority of them anyways).

Part 3: Conditioning--approximately 10 minutes

Note: the suggested times below can be adapted to best fit your own classroom's timing needs. Other alternatives could include fewer trials (see part 5) with longer conditioning time frames.

1. Label two petri dishes, one with ".1 mM drug" and one with "spring water".
2. In the petri dish with .1 mM drug, mimic the planarian's **non-preferred environment (the light)**.
3. Gently move the planarian from the split dish to the newly prepared petri dish and set aside for 2 minutes.
4. In the petri dish with spring water, mimic the planarian's **preferred environment (the dark)** by covering the dish on all sides with black paper.

- After the 2 minutes have passed, move the planarian to the newest dish prepared in step 4, and set aside for an additional 2 minutes.
-

Part 4: Post-Test--*approximately 10 minutes*

- Place the planarian back in the split dish you used in *Part 2: Pre-test (with spring water!)*. Record how much time it spends on the light versus the dark side of the plate over the course of 5 minutes using seconds as your unit.

Time on the Light Side (seconds)	this number should be higher than the number observed in the pretest
Time on the Dark Side (seconds)	this number should be less than the number observed in the pretest

****note:** this assumes that the nonpreferred environment is the light side and the preferred environment is the dark side. If those two are flipped then the data in the above table will also be reversed.

- Compare these results to the data you gathered in the Pre-Test.
 - Subtract the times gathered in the pre-test from the times gathered in the post-test using the spaces below.
 - Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test)=

_____ - _____ = positive number

- Time on the Dark Side (Post-Test)- Time on the Dark Side (Pre-Test) =

_____ - _____ = negative number

****If the differences are positive, that indicates that the planarians have a change in preference for that side following conditioning. If the differences are negative, that would indicate that following conditioning the planarians don't have as strong a preference for that side.**

The positive number should be correlated with the side that was paired with the drug.

Part 5: Further Trials--*approximately 15-20 minutes*

As you increase the drug concentrations, this experiment will shift from rewarding effects of the drugs to aversive effects of the drugs. In this case, some planarians may shift their patterns and begin to side more time on the preferred side compared to with lower drug concentrations. This

part of the experiment should probably only be completed with more advanced classes and if time permits.

1. Repeat Parts 3 and 4 using drug concentrations of 1 mM and 5 mM.
2. Record new Post-Test Times Below:

1 mM Drug Concentration

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

5 mM Drug Concentration

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

3. Calculate new Differences Below:

1 mM Differences:

Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test)=

_____ - _____ = _____

Time on the Dark Side (Post-Test)- Time on the Dark Side (Pre-Test) =

_____ - _____ = _____

5 mM Differences:

Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test)=

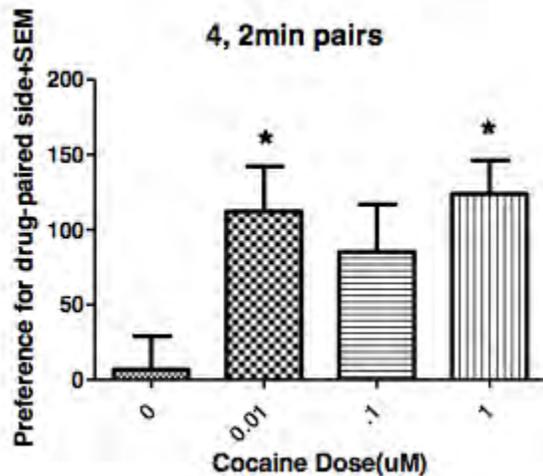
_____ - _____ = _____

Time on the Dark Side (Post-Test)- Time on the Dark Side (Pre-Test) =

_____ - _____ = _____

Part 6: Graphing Your Results--*approximately 5 minutes*

Using your positive differences only, graph the results of your findings for each concentration of drug tested. **You will be using the numbers obtained from the light side of the plate!**



These are the results obtained experimentally in our lab using cocaine, though the results should be similar with other drugs like nicotine or caffeine. It is recommended that you test this out yourself in your classroom before attempting with students as results may vary.

Part 7: Scientific Method Recap--*approximately 10 minutes*

What **question** were we trying to answer with this experiment?

How does exposure to a drug change an organism's preference for a particular environment?

What might have been our **hypothesis**?

A valid hypothesis could predict that exposure to a drug will increase an organism's preference for the environment where the drug was received, decrease the preference for the environment received, or would have no effect on environment preference.

What **methods** did we use to test our hypothesis?

We first observed a planarian's baseline environmental preference, and then conditioned the planarian to receive a drug in its non-preferred environment. We then retested the planarian's environmental preference and calculated the differences between our initial and final results.

What were our **results**?

Following conditioning in its non-preferred environment, the planarian's environmental preference changed.

What **conclusions** can we draw from our results?

The planarian now associated its non-preferred environment with a drug, and therefore wanted to spend more time in that environment in the hopes of receiving more drug.

Part 8: Discussion Questions--approximately 10 minutes

1. In the Pre-Test, what was the planarian's preferred side? What do you think might be a reason why planarians prefer that side?

Most likely, the planarian's preferred side was the dark side. This preference exists because it helps the planarian avoid predators.

2. In the Conditioning phase, what was the stimulus and what was the response?

The stimulus is the drug and the response is the amount of time spent in the drug-paired environment.

3. How were we able to tell that the planarian's preferred environment had changed after conditioning?

We were able to tell that the planarian's preferred environment changed after conditioning because during the pre-test it preferred the dark, but after receiving drug in the light, it started to prefer the light over the dark.

4. In the Conditioning phase of the experiment, we paired the drug with the planarian's non-preferred environment, and gave spring water with the preferred environment. Why might that have changed the planarian's preferences?

The planarian's preference might have changed because it had now created an association between the drug and the light side of the dish. It now is choosing to spend more time in the light in the hopes of getting more drug. Alternatively, the planarian's preferences may have changed because the drug had an adverse effect on the planarian's decision making process, and it no longer is able to correctly choose its safer preferred environment.

5. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. How might this experiment help to explain what happens to humans when they ingest drugs?

Like in planarians, taking drugs can change human behavior and preferences too, as well as cloud judgement.

Rewarding Effects (Conditioned Place Preference)

Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Preferred Environment	The environment an organism chooses to be in when given a choice
Non-preferred Environment	The environment an organism avoids when given a choice
Stimulus	Something that causes a change or reaction
Conditioning	A form of learning involving the formation, strengthening, or weakening of an association between a stimulus and a response

Part 2: Pre-Test

1. Prepare a petri dish that is half covered by black construction paper and fill it with spring water.
2. Gently place a planarian into the petri dish, and record how much time it spends on the light versus the dark side of the plate over the course of 5 minutes. Record your observations below using seconds as your unit.

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

3. Which side of the plate did the planarian spend more time on?
_____ This is the planarian's **preferred environment**.
4. Which side of the plate did the planarian spend less time on?
_____ This is the planarian's **non-preferred environment**.

If you have a planarian whose preferred environment is the DARK, continue to part 3: Conditioning. However if your planarian prefers the light, please repeat part 2 with a different planarian.

Part 3: Conditioning

1. Label two petri dishes, one with “.1 mM drug” and one with “spring water”.
 2. In the petri dish with .1 mM drug, mimic the planarian’s **non-preferred environment (the light)**.
 3. Gently move the planarian from the split dish to the newly prepared petri dish and set aside for 2 minutes.
 4. In the petri dish with spring water, mimic the planarian’s **preferred environment (the dark)** by covering the dish on all sides with black paper.
 5. After the 2 minutes have passed, move the planarian to the newest dish prepared in step 4, and set aside for an additional 2 minutes.
-

Part 4: Post-Test

1. Place the planarian back in the split dish you used in *Part 2: Pre-test (with spring water!)*. Record how much time it spends on the light versus the dark side of the plate over the course of 5 minutes using seconds as your unit.

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

2. Compare these results to the data you gathered in the Pre-Test.
 - a. Subtract the times gathered in the pre-test from the times gathered in the post-test using the spaces below.
 - b. Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test) =
_____ - _____ = _____
 - c. Time on the Dark Side (Post-Test) - Time on the Dark Side (Pre-Test) =
_____ - _____ = _____

**If the differences are positive, that indicates that the planarians have a change in preference for that side following conditioning. If the differences are negative, that would indicate that following conditioning the planarians don't have as strong a preference for that side.

Part 5: Further Trials

1. Repeat Parts 3 and 4 using drug concentrations of 1 mM and 5 mM.
2. Record new Post-Test Times Below:

1 mM Drug Concentration

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

5 mM Drug Concentration

Time on the Light Side (seconds)	
Time on the Dark Side (seconds)	

3. Calculate new Differences Below:

1 mM Differences:

Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test) =

_____ - _____ = _____

Time on the Dark Side (Post-Test) - Time on the Dark Side (Pre-Test) =

_____ - _____ = _____

5 mM Differences:

Time on the Light Side (Post-Test) - Time on the Light Side (Pre-Test) =

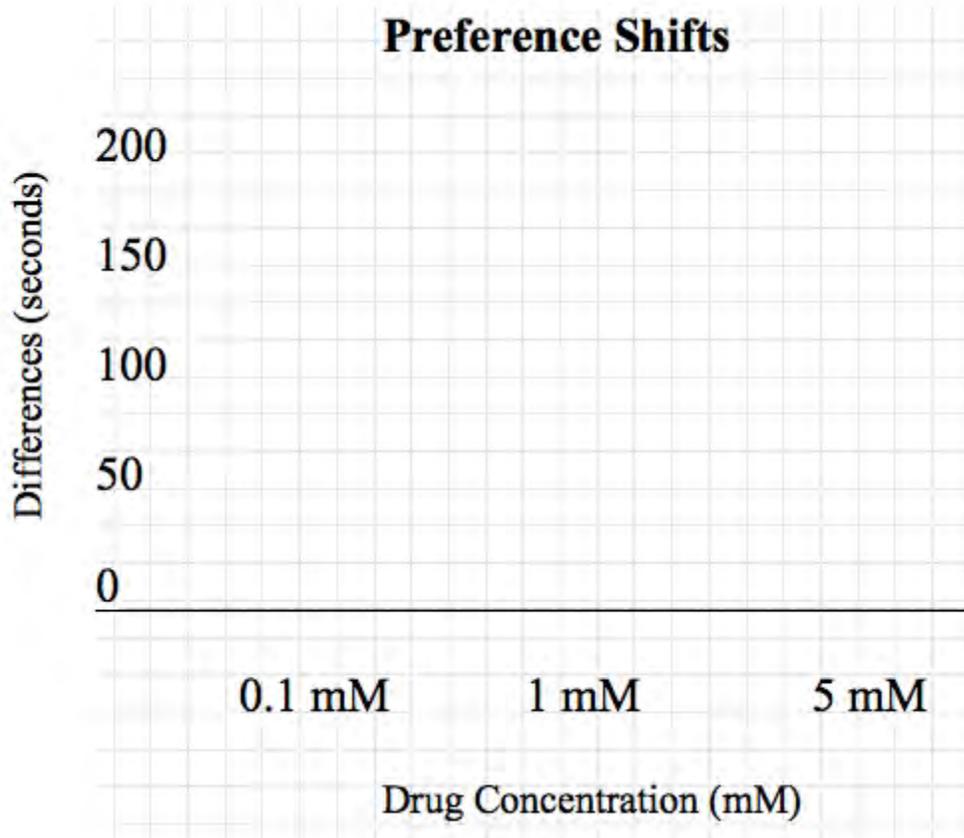
_____ - _____ = _____

Time on the Dark Side (Post-Test) - Time on the Dark Side (Pre-Test) =

_____ - _____ = _____

Part 6: Graphing Your Results

Using your positive differences only, graph the results of your findings for each concentration of drug tested.



Part 7: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 8: Discussion Questions

1. In the Pre-Test, what was the planarian's preferred environment? What do you think might be a reason why planarians prefer that side?
2. In the conditioning phase, what was the stimulus and what was the response?
3. How were we able to tell that the planarian's preferred environment had changed after conditioning?
4. In the conditioning phase of the experiment, we paired the drug with the planarian's non-preferred environment, and gave spring water with the preferred environment. Why might that have changed the planarian's preferences?
5. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. How might this experiment help to explain what happens to humans when they ingest drugs?

Teacher's Guide: Drug-Drug Interactions using Acute Stereotypy-- **50-55 Minutes Total**

Goal: Abuse of a single drug or addictive substance is the exception rather than the rule. Most drug abusers consume multiple drugs, and the presence of multiple drugs in the body can increase their risk of producing addictive effects and toxicity. Some of the more common drug combinations taken by humans are speedballs (heroin + cocaine) and combinations of cocaine and alcohol. For cocaine-dependent patients, up to 90% receiving inpatient treatment and 50% receiving outpatient treatment are also dependent on ethanol. Ethanol counters anxiety precipitated by cocaine withdrawal but also facilitates cocaine craving that increases relapse rates. Further, promising anti-cocaine medications, notably modafinil, are often less effective in patients who simultaneously abuse cocaine and ethanol.

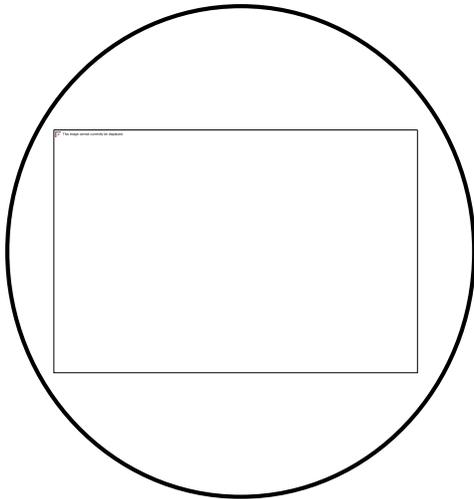
Frappuccinos and energy drinks such as Monster are also commonly consumed by the United States population. These beverages contain significant amounts of caffeine and sugar and it is possible that the rewarding and stimulant effects of caffeine are increased in the presence of sugar. Our experiments here are designed to test effects of a caffeine/sugar combination on planarian activity. The lesson could also be coupled with lessons 5 and 6 to assess effects of caffeine/sugar combinations on anxiety and depression.

Part 1: Vocabulary-- *approximately 5-10 minutes*

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Acute	A response of rapid onset and/or short duration
Stereotypy	Repetitive or ritualistic movement (Ex: Body rocking, C-Shaped Movements)
Synergism	When two drugs produce an effect together that is greater than the sum of their individual effects ($1+1 = \text{more than } 2$)
Potentiation	When one drug that may not have much of an effect on its own boosts the effects of another drug ($1+0 = \text{more than } 1$)
Antagonism	When one drug reduces or blocks the effects of the other ($1+1 = \text{less than } 1$)

Part 2: Observation-- Approximately 10 minutes

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.
2. Draw a picture in the circle below of what you saw under the microscope as well as list your observations. **Under observations students should note color, movement, size, shape, etc. It is recommended that the microscopes are set up for the students.**



Part 3:

Experiment-- Approximately 20 minutes

How is a planarian affected by caffeine, sucrose, and a mixture of the two?

CAFFEINE

1. Label a petri dish with “5.0 mM Caffeine”.
2. Place your planarian on a petri dish with 5.0 mM of Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back in a petri dish with Spring Water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

At this concentration (5.0 mM caffeine), an average of ~10 C-Shapes is expected

SUCROSE

1. Label a petri dish “1% Sucrose”
2. Place your planarian on a petri dish with 1% Sucrose.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

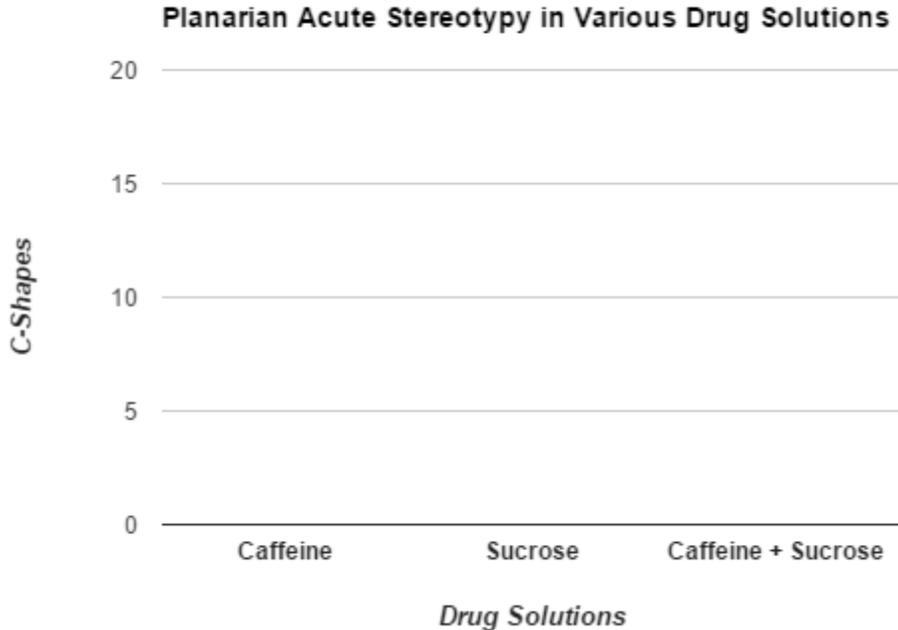
At this concentration (1% sucrose), an average of 0-5 C-Shapes is expected (sucrose will not really produce much of an effect on its own!)

CAFFEINE + SUCROSE

1. Label a petri dish “1% Sucrose + 5.0 mM Caffeine”
2. Place your planarian in a petri dish with 1% Sucrose and 5.0 mM Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

At this concentration (5.0 mM caffeine + 1% sucrose), an average of >10 C-Shapes is expected
 Fill in the graph with your results below. (Use the averages!)



Caffeine should be ~10, Sucrose ~0, and Caffeine + Sucrose should be >10

Notes: You can vary the experiments in the following ways: 1) test different concentrations of a fixed concentration of one substance (e.g. 1% sucrose) with different concentrations of the second substance (e.g. 0.1, 1, 5 mM caffeine); 2) test different drug combinations such as caffeine + sugar; alcohol + sugar; alcohol + caffeine; etc.

Part 4: Scientific Method Recap-- Approximately 10 minutes

What **question** were we trying to answer with this experiment?

Will caffeine, sucrose, or a combination of the two produce more C-shapes in the planarian?

What might have been our **hypothesis**?

Variable; whatever the students hypothesize will produce the greatest effect

What **methods** did we use to test our hypothesis?

Placing planarians in three different drug solutions for three trials apiece, counting the C-shapes elicited, and taking the averages

What were our **results**?

Ideally, the students should see that caffeine produces an effect, sucrose does not, and the combination of the two produces a greater effect than caffeine. (Potentiation!)

What **conclusions** can we draw from our results?

Caffeine elicits stereotypy while sucrose does not, but when put together, they produce an even greater effect than caffeine alone. Drug interactions can alter the typical behavior of drugs, etc.

Part 5: Discussion Questions-- Approximately 10 minutes

1. What affect did caffeine alone have on the planarian?

It elicited C-shapes

2. What affect did sucrose alone have on the planarian?

It failed to elicit C-shapes

3. Which drug solution elicited the most C-shapes from the planarian?

The caffeine + sucrose should elicit the most

4. What type of drug-drug interaction would you classify caffeine + sucrose as (synergism, potentiation, or antagonism)? Why?

We should see potentiation here, because the interaction takes the sucrose from having no effect to having an enhancing effect on the caffeine. However, results may vary

5. What are some examples of beverages that we as humans consume that would fall under the following categories:

a. Sucrose: **gatorade, kool aid, etc.**

b. Caffeine: **coffee, tea, mio, etc.**

c. Sucrose + Caffeine: **frappuccinos, monster energy drinks, red bull, etc.**

6. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how the different beverages you listed above affect our own bodies?

Substances we take into our bodies can interact and produce a greater effect than they would individually, or that we would expect them to.

Drug-Drug Interactions using Acute Stereotypy

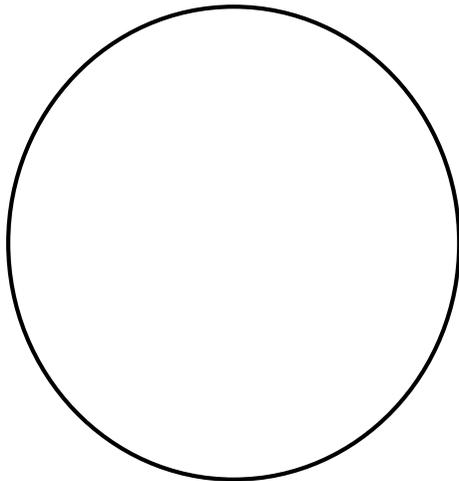
Part 1: Vocabulary

Word	Definition
Planarian	A kind of flatworm, and the stars of this experiment!
Solute	The minor component in a solution, dissolved in the solvent
Solvent	The major component in a solution, what the solute is dissolved in
Solution	A liquid mixture where the solute is dissolved in the solvent (Ex: Salt Water, when you have dissolved salt in water, Salt here is acting as the solute and water is acting as the solvent)
Acute	A response of rapid onset and/or short duration
Stereotypy	Repetitive or ritualistic movement (Ex: Body rocking, C-Shaped Movements)
Synergism	When two drugs produce an effect together that is greater than the sum of their individual effects ($1+1 = \text{more than } 2$)
Potentiation	When one drug that may not have much of an effect on its own boosts the effects of another drug ($1+0 = \text{more than } 1$)
Antagonism	When one drug reduces or blocks the effects of the other ($1+1 = \text{less than } 1$)

Part 2: Observation

1. Place the planarian in a petri dish of spring water using the disposable pipettes. Using the microscope, observe the planarian under the microscope.

2. Draw a picture in the



circle
below
of which
at you
saw
under
the
microscope

as well as list your observations.

Part 3: Experiment--

How is a planarian affected by caffeine, sucrose, and a mixture of the two?

CAFFEINE

1. Label a petri dish with "5.0 mM Caffeine".
2. Place your planarian on a petri dish with 5.0 mM of Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back in a petri dish with Spring Water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

SUCROSE

1. Label a petri dish "1% Sucrose"
2. Place your planarian on a petri dish with 1% Sucrose.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

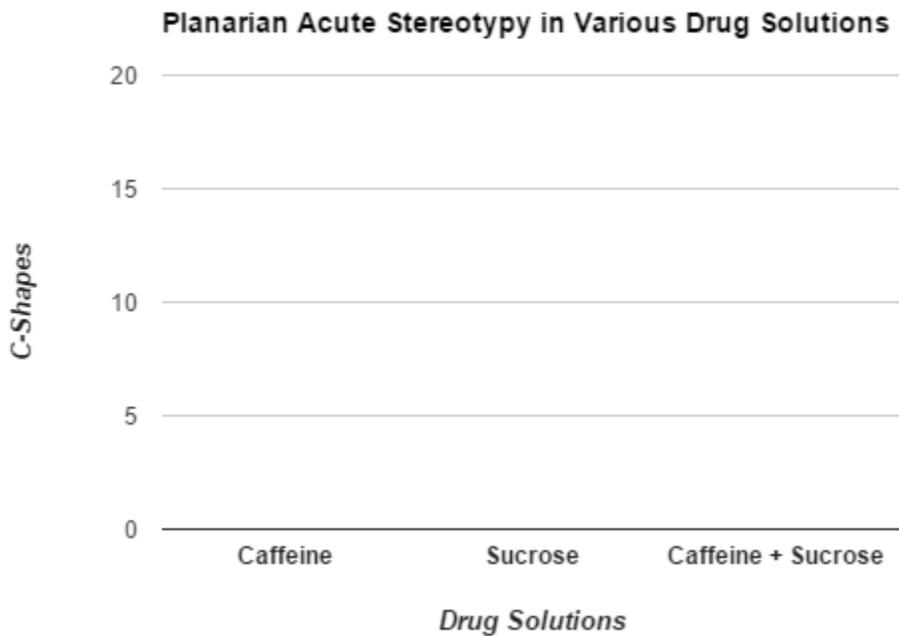
	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

CAFFEINE + SUCROSE

1. Label a petri dish "1% Sucrose + 5.0 mM Caffeine"
2. Place your planarian in a petri dish with 1% Sucrose and 5.0 mM Caffeine.
3. How many times does it exhibit C-Shaped behavior in 2 minutes (when the planarian moves its body into a C-Shape and then back to normal)?
4. When finished, place your planarian back into a petri dish with spring water.
5. Repeat steps 2-4 an additional 2 times with different planarians.

	Trial 1	Trial 2	Trial 3	Average
C-Shapes				

Fill in the graph with your results below. (Use the averages!)



Part 4: Scientific Method Recap

What **question** were we trying to answer with this experiment?

What might have been our **hypothesis**?

What **methods** did we use to test our hypothesis?

What were our **results**?

What **conclusions** can we draw from our results?

Part 5: Discussion Questions

1. What affect did caffeine alone have on the planarian?
2. What affect did sucrose alone have on the planarian?
3. Which drug solution elicited the most C-shapes from the planarian?
4. What type of drug-drug interaction would you classify caffeine + sucrose as (synergism, potentiation, or antagonism)? Why?
5. What are some examples of beverages that we as humans consume that would fall under the following categories:
 - a. Sucrose:
 - b. Caffeine:
 - c. Sucrose + Caffeine:

6. Part of what makes planarians so cool is that they have a brain and a central nervous system that is a lot like ours. What might this mean about how the different beverages you listed above affect our own bodies?

This document serves as a list of ideas to expand upon and mix-up the experiments outlined here to best fit the needs of your classroom. Most of these ideas have come from teachers who have tried out these methods in their own classrooms and had success. Please feel free to pick and choose as well as alter and add to this list!

- **Music Lyrics**

So much of today's music centers around drugs and drug culture. Have your students find a set of lyrics dealing with drugs or addiction, and discuss the themes present in these songs. Use these lyrics to prompt class discussions about drugs and addiction.

- **Act Out Plays**

Use the students as your demonstrations to explain how neural transmission works. Line students up and have them act out neurons, neurotransmitters, and other various signals. You can also use these demonstrations to show the class what changes when drugs are present, as well as receptor up/down regulation, tolerance, sensitization, etc. etc.

- **Research Articles**

Use the topics of drugs and addiction to have your students read primary source research articles on the topic. Have them read a paper and write up a report to get a feel for the kinds of questions scientists are asking on these topics. You can use these exercises to help students take ownership of their own future planarian experiments in lessons to come. (Note: There is a large database of sample articles already present on the SEADAP googledrive)

- **Adapt Experiments with Multiple Species of Planarians**

Any of the lessons outlined here can be adapted to compare the effects of these drugs on multiple different species of planarians. SEADAP can connect you with up to 3 different species of planarians to have your students compare similarities and differences between species.

- **Compare effects of sugar with sugar substitutes**

Any of the lessons outlined here can be adapted to compare the effects of sucrose with those of sugar substitutes including Splenda, Equal, Sweet'N Low, and Saccharin. SEADAP can include kits with these sugar substitutes if you choose this experiment.

Tips for Success

1. How do you get the planarian off of the scoopula?
 - a. A lot of it is just trial and error, but some teachers have found that using a paintbrush helps. Others found that sucking planarians up through a pipette also worked.
2. What do I do when I break a planarian?
 - a. The good news with planarians is that they regenerate, so no worries, but we recommend that each group of students do their experiments multiple times with different planarians so that if this happens they still will be able to gather data.
3. What do I do with outlier results?
 - a. We like to build as many trials into each experiment as possible to help address this issue. You can either use the outlier results as a teaching moment to talk about things like human error and differences in environmental conditions, or you can simply throw the outlier results out to have more cohesive class results and emphasize a main point.
4. Note: Some of the higher doses of drugs can be lethal to planarians and can on occasion kill them.
 - a. You can and should adapt drug concentrations to the needs of your classroom. Feel free to use the higher doses and help make the connections between high doses of drugs and the health of an organism, or choose to stay in lower concentrations.
5. Many teachers found it was helpful to assign student roles within experimental groups to help keep all the students engaged.
 - a. Example roles: Time Keeper, Note Taker, Group Leader, Transporter, etc.
6. Some teachers also have found that adding food coloring to the various drug solutions helps students keep each one straight



SEADAP

SCIENCE EDUCATION AGAINST DRUG ABUSE PARTNERSHIP

What is a Planarian?

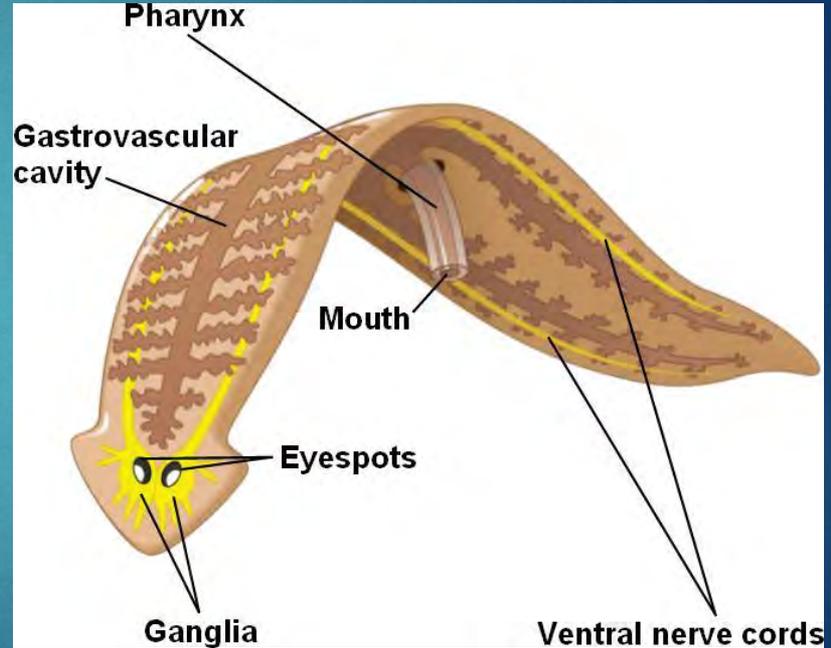


- Flatworms (phylum)
- Invertebrates
- Bilateral symmetry
- Mammalian-like behavioral responses
- Responsive to addictive substances
- Preference for dark places

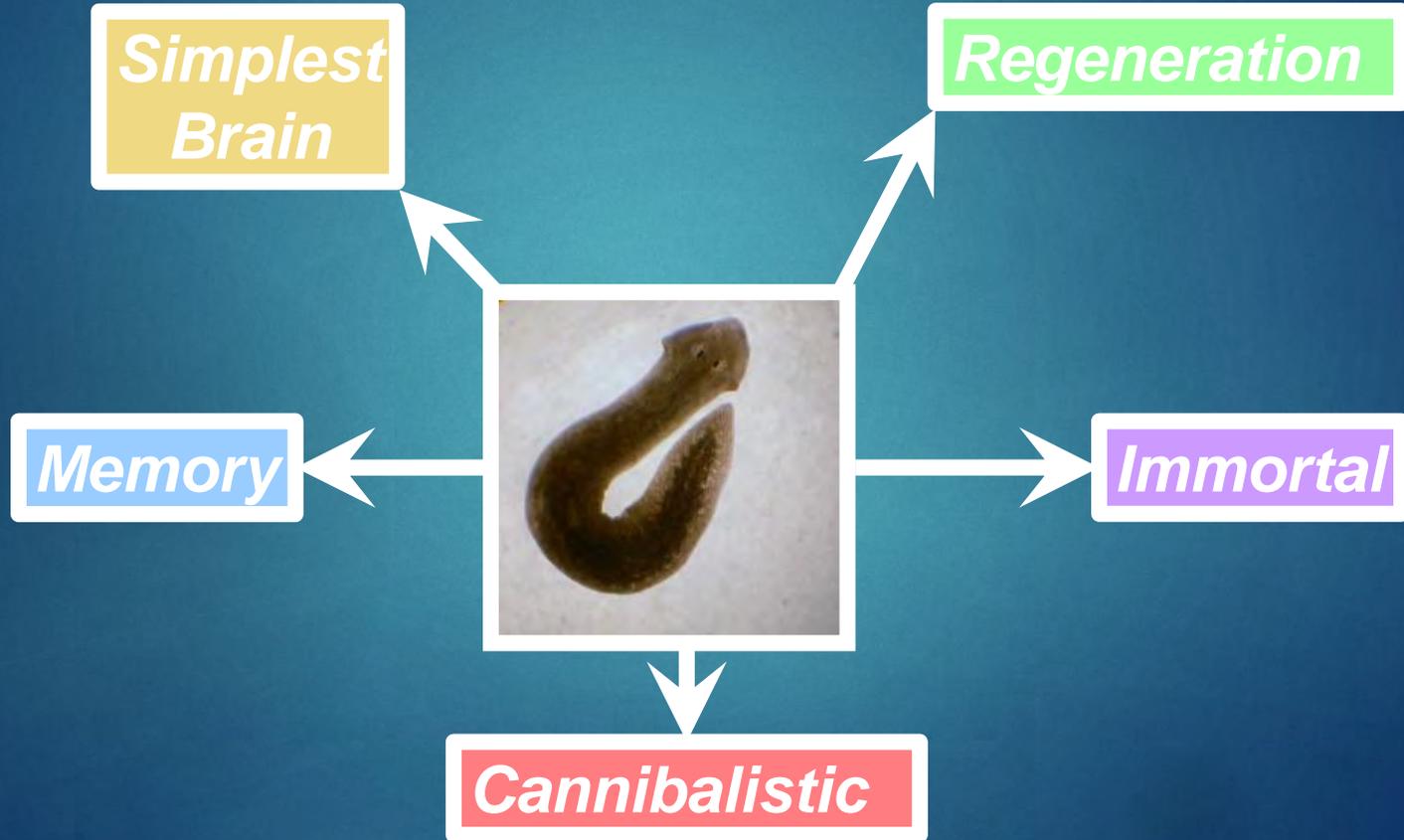
<i>Planaria</i>	
Scientific classification	
Kingdom:	Animalia
Phylum:	Platyhelminthes
Class:	Turbellaria
Order:	Tricladida
Suborder:	Continenticola
Family:	Planariidae
Genus:	<i>Planaria</i>

Planarian Anatomy

- **Pharynx**- the muscular mouth
- **Eyespots**- primitive eyes that contain photoreceptors
- **Photoreceptors**- receptors in the eye that respond to light
- **Phototaxis**- movement where an organism moves towards or away from light
- **Ganglia**- a bundle of nerve fibers

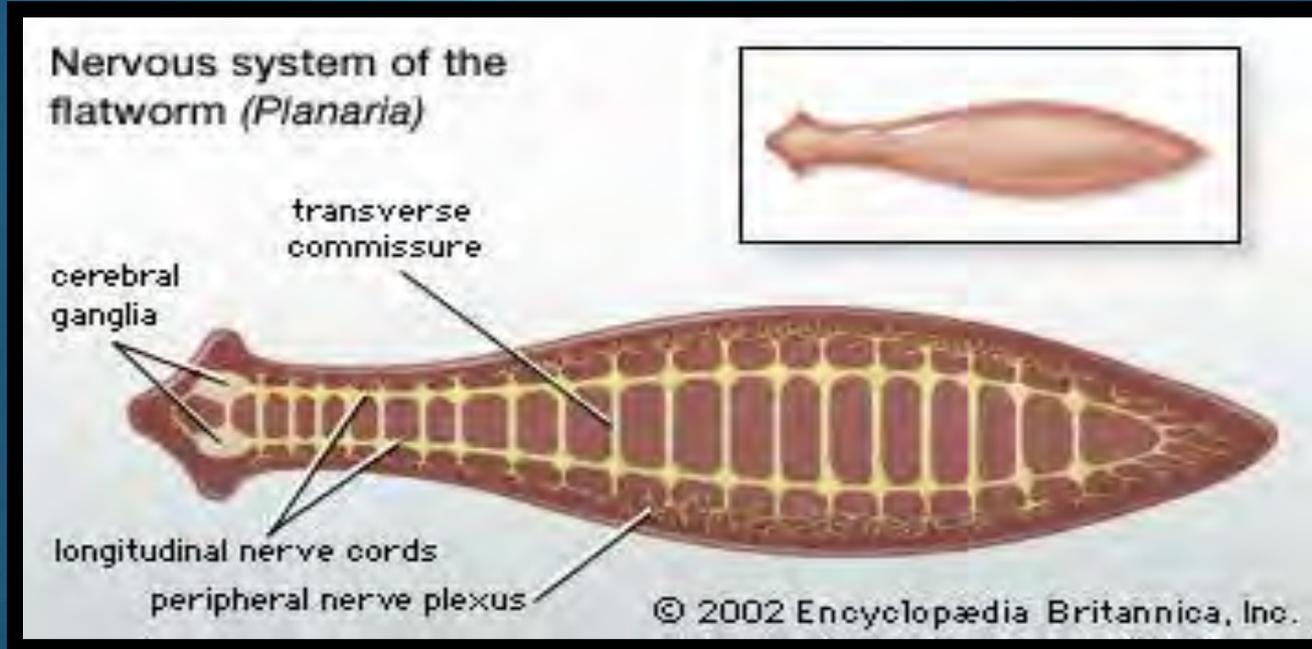


Planarian Characteristics



Planarian Nervous System

*Simplest
Brain*



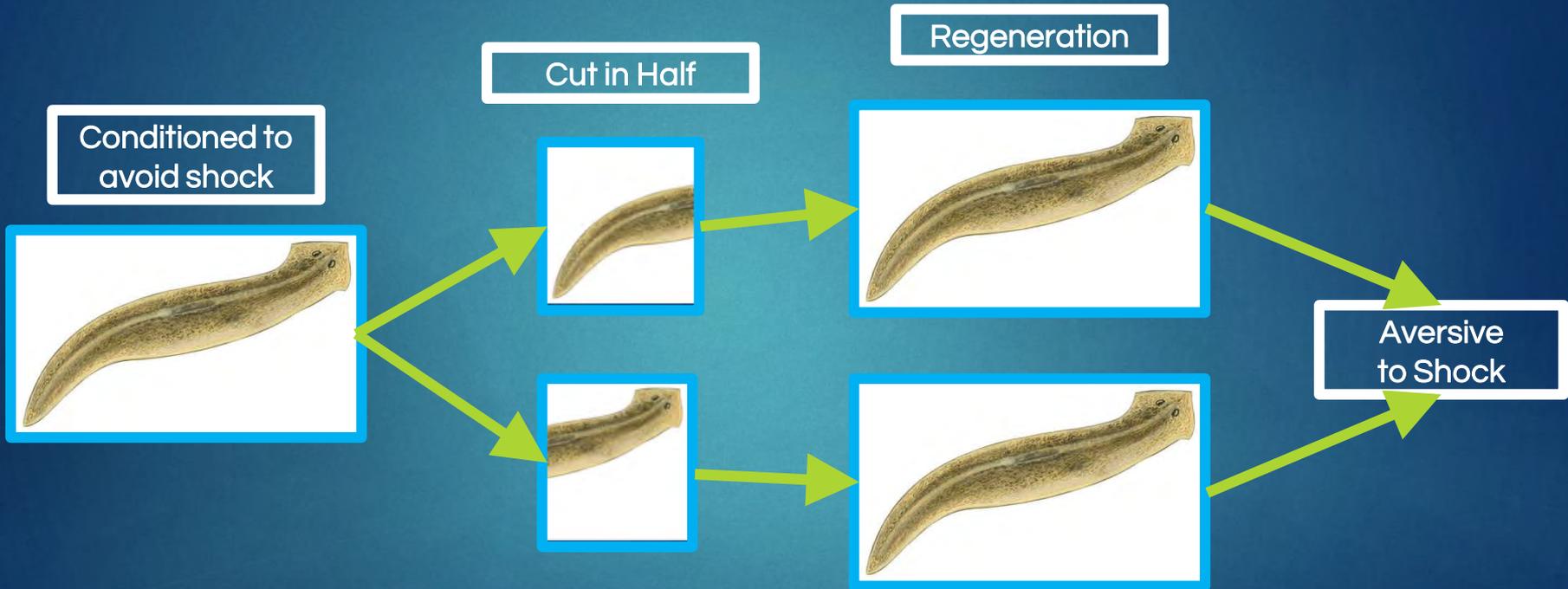
Immortal

Regeneration



- If a planarian is cut into several pieces, each piece will regenerate into a complete organism in ~8 days
- **Neoblasts**- pluripotent stem cells inside the planarian that can become any cell type
- Planarians are said to be immortal because of their high cell turnover rate and ability to regenerate

Memory



Robert Thompson and James V. McConnell (1955)

Cannibalistic

*If they don't
feed us soon*

- Planarians do not need to feed very often
- However, if food is not available, they are known to be cannibalistic and eat one another





Planarians as a Model for Addiction

**Addictive Substances
(Drug and Natural Reinforcers)**



**Motor
Effects**

**Tolerance and
Sensitization**

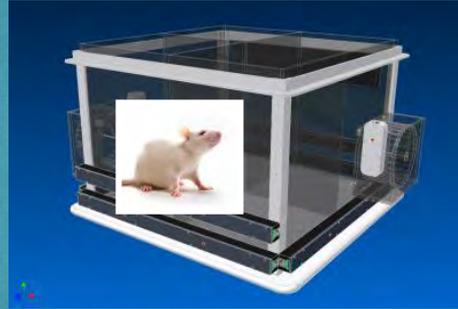
**Withdrawal and
Dependence**

**Reward and
Reinforcement**

Anxiety

Motor Effects

Most drugs that produce rewarding and reinforcing (*i.e.*, addictive) effects in humans produce an increase in locomotor activity when administered to animals. The locomotor activity can be divided into stereotypical activity and ambulatory activity.



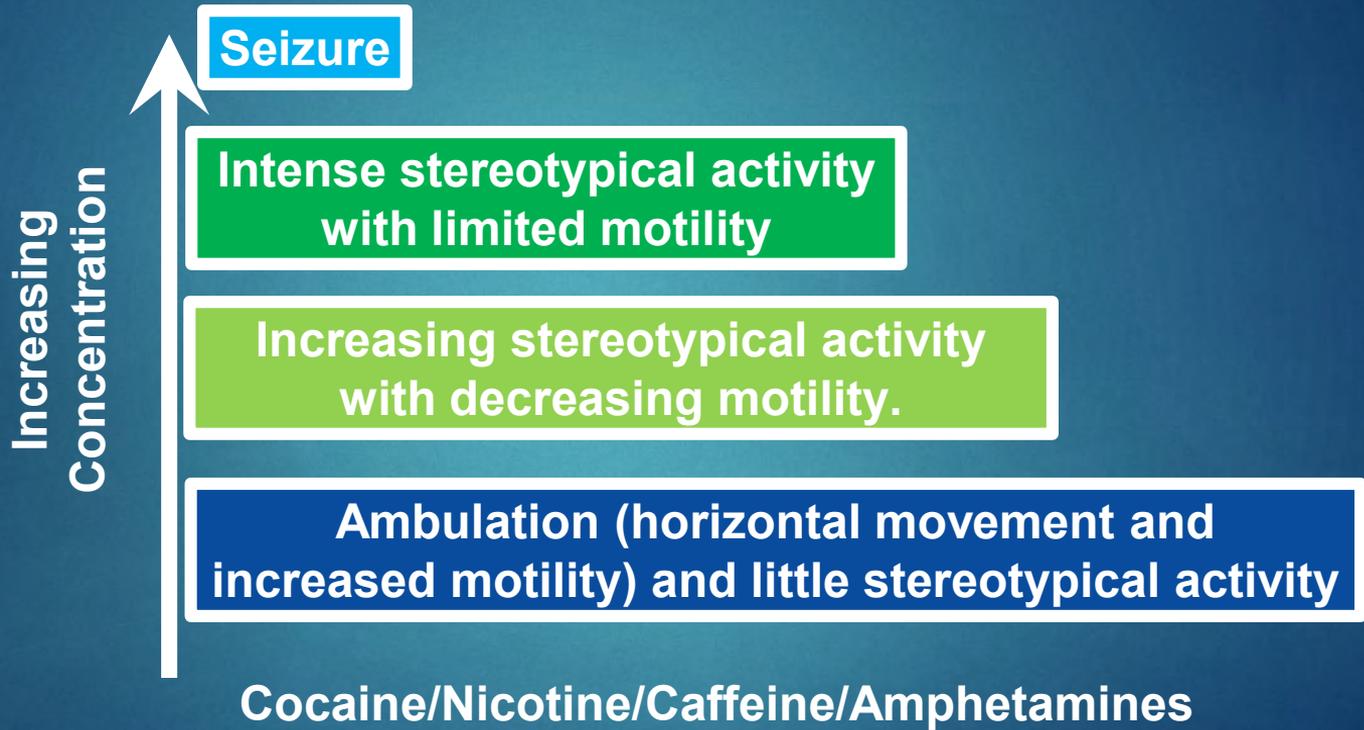
***Stereotypical
Activity***

***Ambulatory
Activity
(Motility)***

Examples in Humans and Planarians

Organism	Stereotypy	Motility
Humans	Body rocking, crossing and uncrossing of legs, tweaking	Walking, running, etc.
Planarians	Head bobbing, C-shapes	Swimming with progress

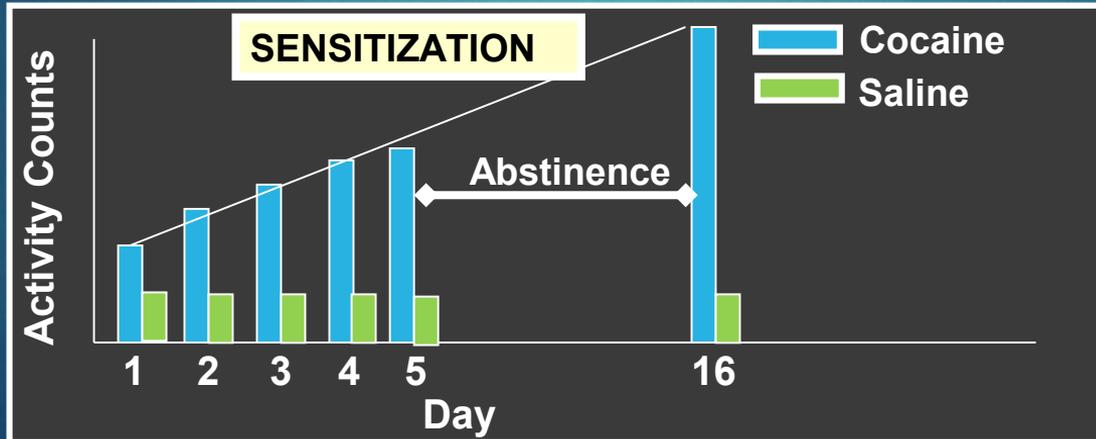
Stereotypical Activity and Motility: impacts of drug concentration



Sensitization vs. Tolerance

Behavioral sensitization is a progressive, enduring increase in response that occurs when an animal is exposed repeatedly to a drug, withdrawn from drug, and then reintroduced to drug.

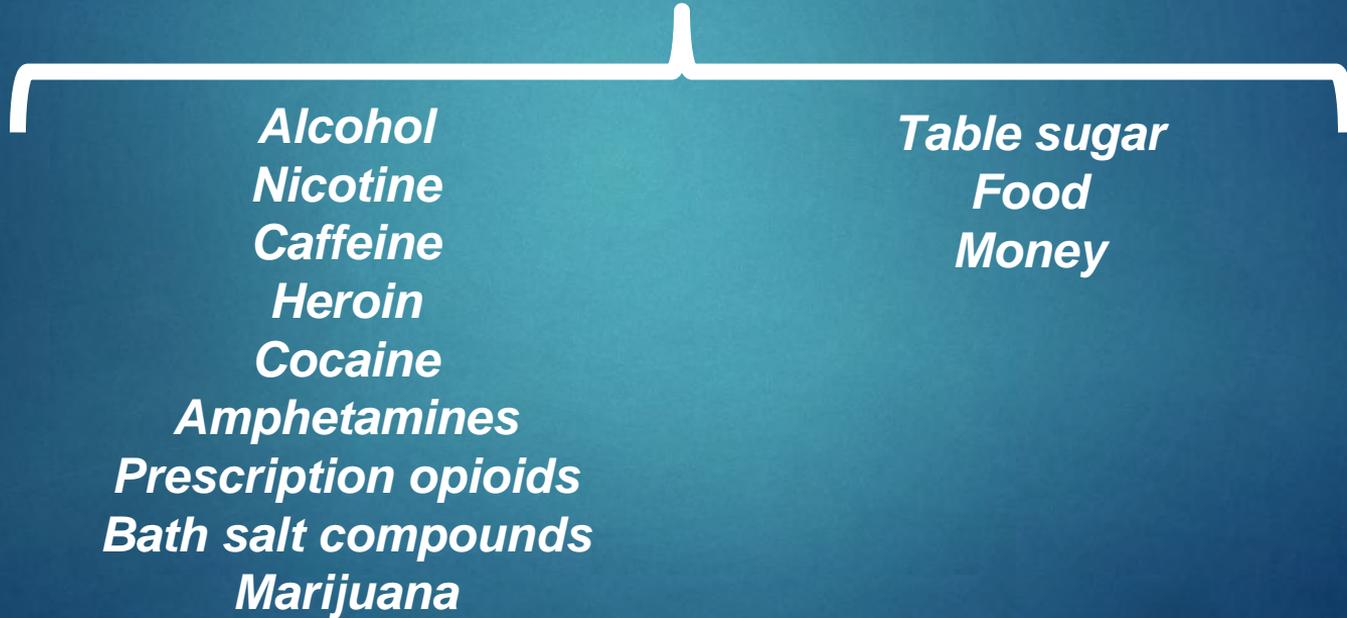
- *Sometimes called 'reverse tolerance'.
- *Most drugs of abuse produce behavioral sensitization.
- *Sensitized response reflects cellular changes during drug exposure that underlies intensification of craving in actual addicts.



Rewarding and Reinforcing Effects

Drug Reinforcer

Natural Reinforcer



Psychological vs. Physical Dependence

Psychological Withdrawal Examples	Physical Withdrawal Examples
anxiety	nausea
dysphoria	vomiting
anhedonia	headaches
depression	tremors

Withdrawal: Signs and Symptoms

Mice

- “Wet-dog” shakes
- Lacrimation
- Teeth Chattering
- Eye Blinking
- Diarrhea
- Escape Behavior
- Weight loss

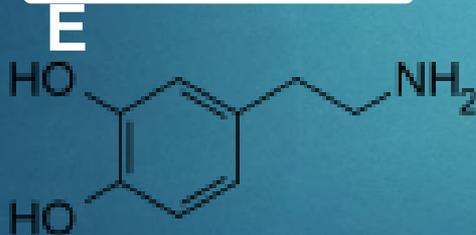
Humans

- Depression
- Irritability
- Anxiety
- Agitation
- Runny nose
- Yawning
- Insomnia
- Weight loss

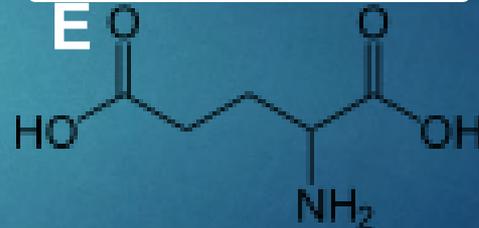
Addiction and the Brain

Dopamine and glutamate: brain neurochemicals responsible for addictive effects of drugs of abuse

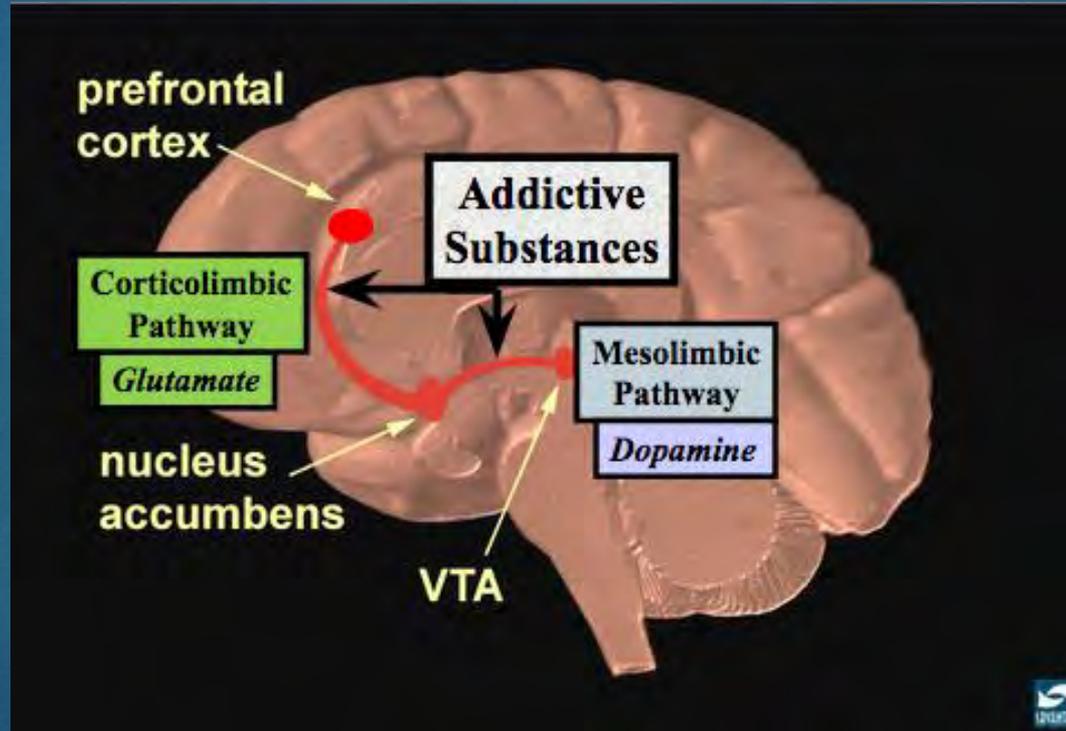
DOPAMIN



GLUTAMAT



Dopamine and Glutamate Pathways



Receptors and Downregulation

- To activate a response in the cell, the drug (ligand) must bind to the receptor
- Constant exposure to the drug (ligand) tells the cell that it doesn't need as many receptors to activate a response



- As a result, the cell makes less receptors
- When there are less receptors, a higher dose of the drug is necessary to get the same effect



Substance Abuse and Drug Addiction

Substance Abuse

A patterned use of a substance in which the user consumes the substance in amounts that are harmful to themselves or others.



Drug Addiction

A *chronic, relapsing brain disease* characterized by compulsive drug seeking and use despite harmful consequences.

Video—*Addiction and the Brain*



Drugs of Abuse

Classification of Drugs

- **Stimulants**

- Elevate mood, energy, alertness, and feelings of well-being
- E.g. cocaine, nicotine, caffeine, MDMA (ecstasy)

- **Depressants**

- Slow normal brain function
- E.g. alcohol, Xanax (anti-anxiety), valium

- **Hallucinogens**

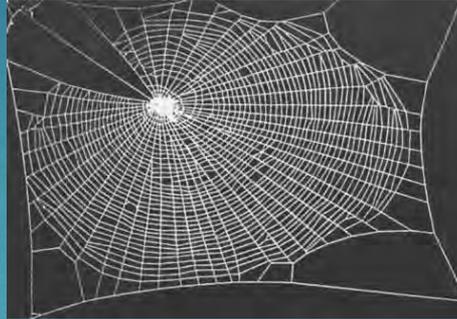
- Alter perception of reality and feeling
- E.g. LSD, MDMA (ecstasy), marijuana

- **Opiates**

- Powerful painkillers
- E.g. heroin, morphine, OxyContin



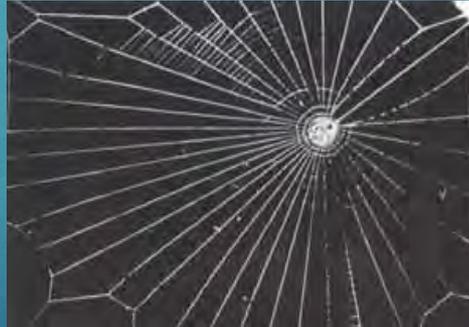
Pharmacology of Drugs Across Species



Normal Spider Web



**CAFFEINE
Treatment**



**LSD
Treatment**

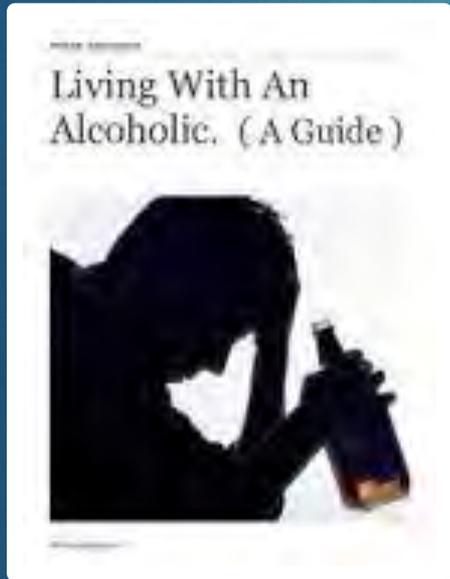


**CANNABIS
Treatment**



Manifestation of Addiction in Humans

Drug Addiction Criteria and Characteristics



Toleranc

Withdrawal
Syndrome

Dependence

Craving

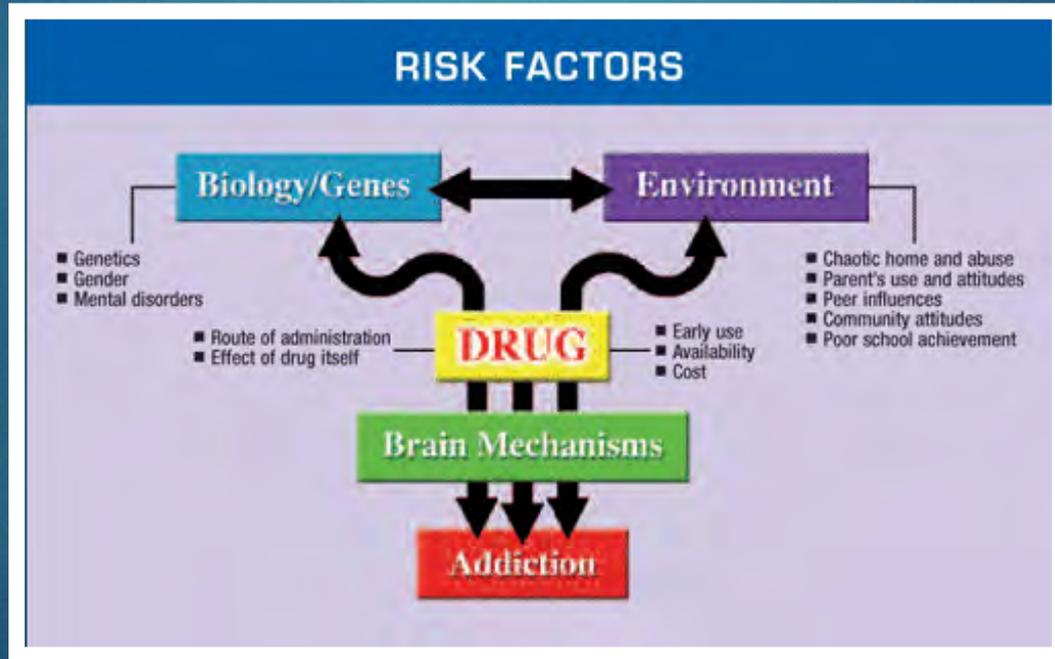
Anxiety

Anhedonia

Depression

Relapse

Risk Factors for Abusing Drugs



Factors Affecting Relapse



- Addictive potential
- Triggers
- Prevention

► Addictive potential (Nutt et al., Lancet 2007)

Drug	Mean	Pleasure	Psychological Dependence	Physical Dependence	Addictive Potential
Heroin	3.0	3.0	3.0	3.0	9.0
Cocaine	2.37	3.0	2.8	1.3	7.1
Nicotine	2.23	2.3	2.6	1.8	6.7
Alcohol	1.93	2.3	1.9	1.6	5.8
Barbiturates	2.10	2.0	2.2	1.8	6.0
Benzodiazepines	1.83	2.1	2.1	1.8	6.0
Amphetamine	1.67	1.9	1.9	1.1	4.9
LSD	1.23	1.1	1.1	0.3	2.5
Cannabis	1.51	1.7	1.7	0.8	4.2
Ecstasy	1.13	1.2	1.2	0.7	3.1

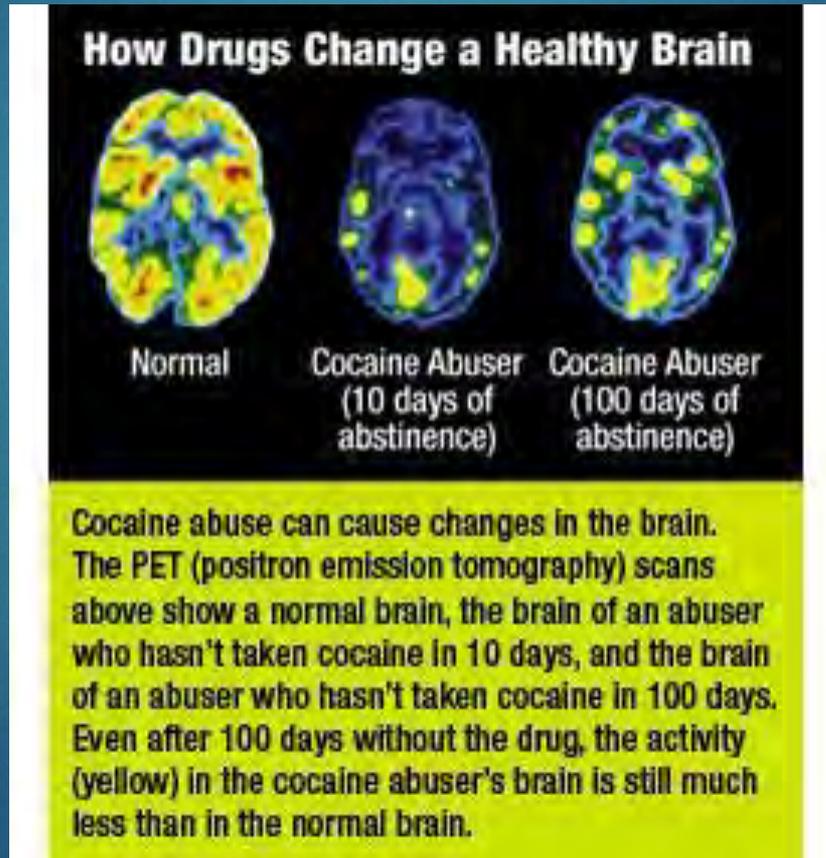
▶ Triggers

- **Environmental triggers**
 - Loud noises, social events
- **Re-exposure triggers**
 - Circumstances that bring the user into proximity of the drug of abuse
- **Stress triggers**
 - Anger, fear, sadness

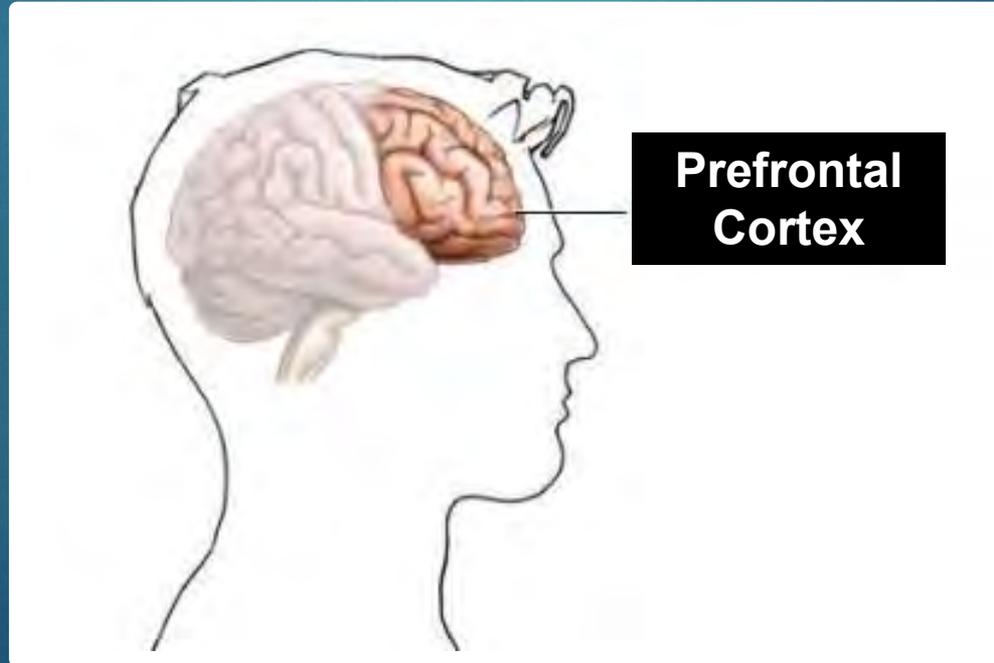
▶ Prevention

- **Medical therapy**
 - Usually targets the receptor sites for the drug
 - Antagonizes the receptor site to prevent dopamine release
 - Why is this a good idea? *Think back to downregulation*
- **Psychological Therapy**
 - Helps the user to identify relapse triggers and the consequences of a relapse
 - Cue exposure is sometimes used to expose the user to a trigger without the “reward” of the drug
 - Helps to reduce the association between the trigger and the drug
- **Contingency Therapy**
 - Focuses on the consequences of a relapse
 - Often uses rewards for the user abstaining from drugs
 - E.g. a voucher that they can purchase retail items with

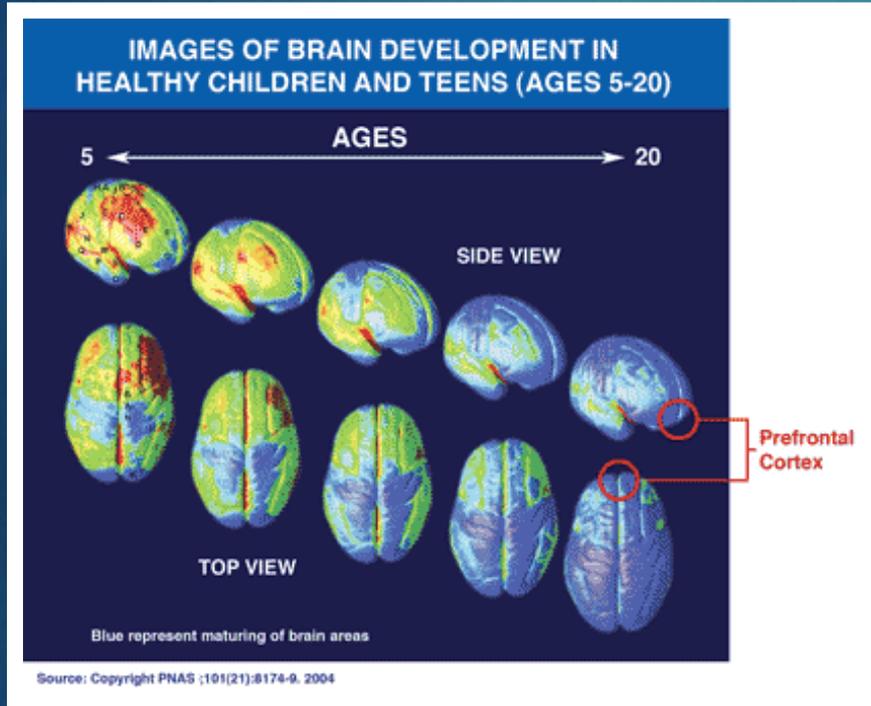
Chronic Drug Abuse Causes Persistent Changes in Brain Activity



Prefrontal Cortex (PFC) contributes to drug-taking by children and adolescents



Prefrontal Cortex and Substance Abuse



One brain area still maturing during adolescence is the ***prefrontal cortex***, the part of the brain that enables us to assess situations, make sound decisions, and keep our emotions and desires under control. The fact that this critical part of an adolescent's brain is still a work in progress puts them at increased risk for making poor decisions (such as trying drugs or continuing to take them). Introducing drugs during this period of development may cause brain changes that have long-lasting consequences.

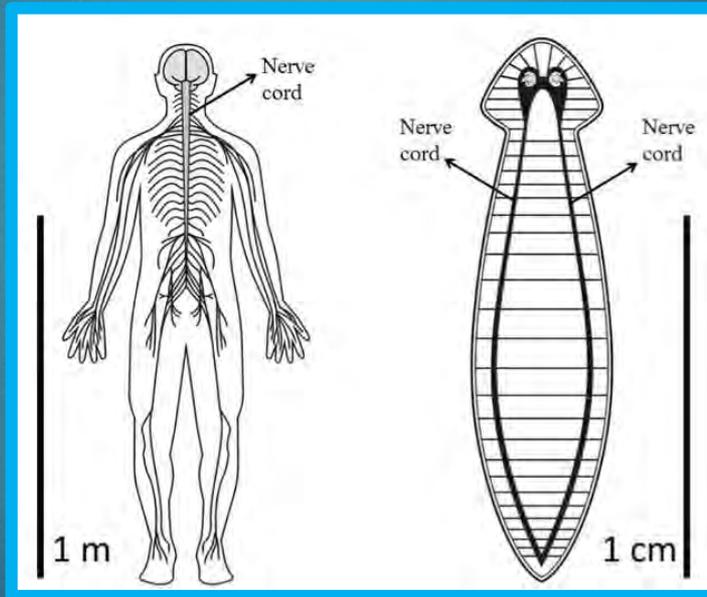


Why and how we use Planarians

Humans vs. Planarians

- Humans

- GABA, glutamate, and other neurotransmitters
- Brain and ganglia
- Drug seeking and withdrawal behaviors
- Ability to become addicted to drugs



- Planarians

- GABA, glutamate, and other neurotransmitters
- Simple brain and ganglia
- Drug seeking and withdrawal behaviors
- Ability to become addicted to drugs

Planarians and Experiments: the “n”

- Planarians are an animal model
 - Outliers
 - Power
 - Drug seeking and withdrawal behaviors
 - Ability to become addicted to drugs

Planarians in Experimentation; the n=

We add

Which Substances Can We Test?

**TABLE SUGAR
(SUCROSE)**



NICOTINE



ALCOHOL



CAFFEINE



Nicotine Classification

NICOTINE

```
graph TD; A[NICOTINE] --> B[Central Nervous System (CNS)  
Psychomotor Stimulant]; B -- produces --> C[Excitement  
Euphoria  
Decreased Fatigue  
Increased Motor Activity];
```

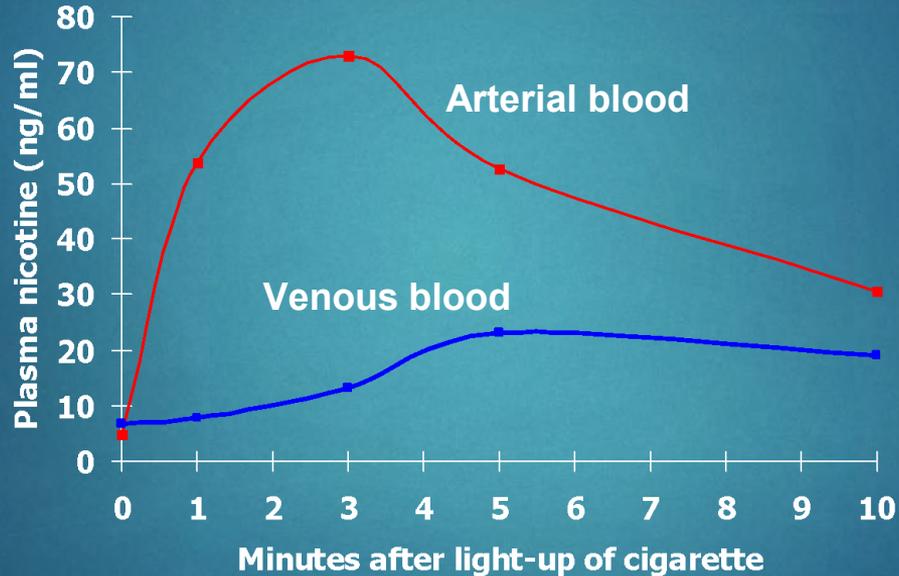
**Central Nervous System (CNS)
Psychomotor Stimulant**

produces

**Excitement
Euphoria
Decreased Fatigue
Increased Motor Activity**

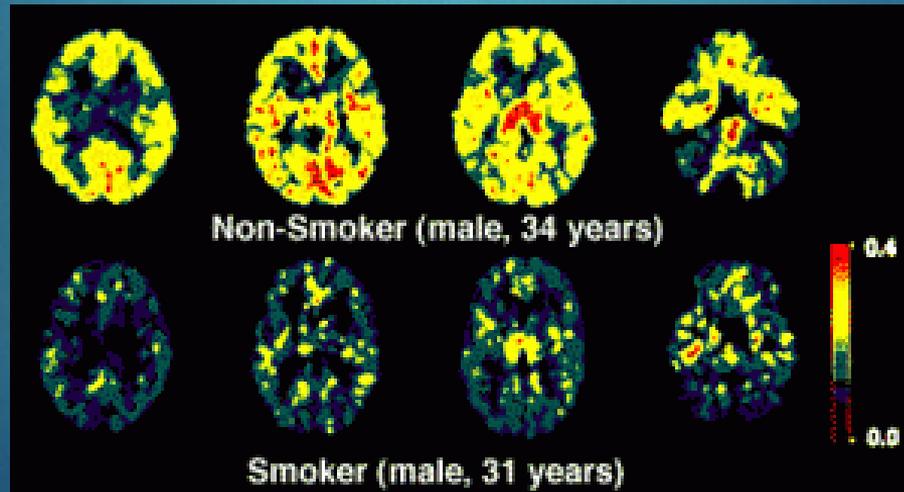
Nicotine Distribution

Nicotine reaches the brain within 11 seconds.



Nicotine Causes Loss of Brain Function

Men who smoke experience more rapid decline in brain function compared with men who did not smoke. This cognitive decline occurs about 10 years earlier.



Tobacco "Addiction"

It is not just nicotine that is responsible for physical and psychological dependence!



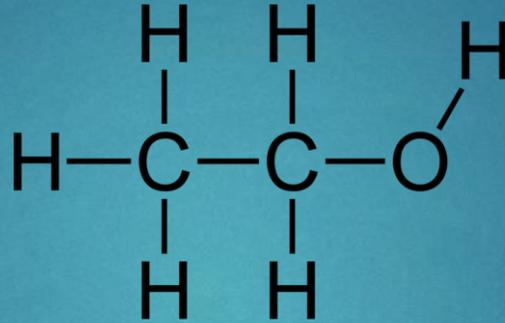
Nicotine

*β -carboline
compounds
(harmine)
(norharman)*

Acetaldehyde

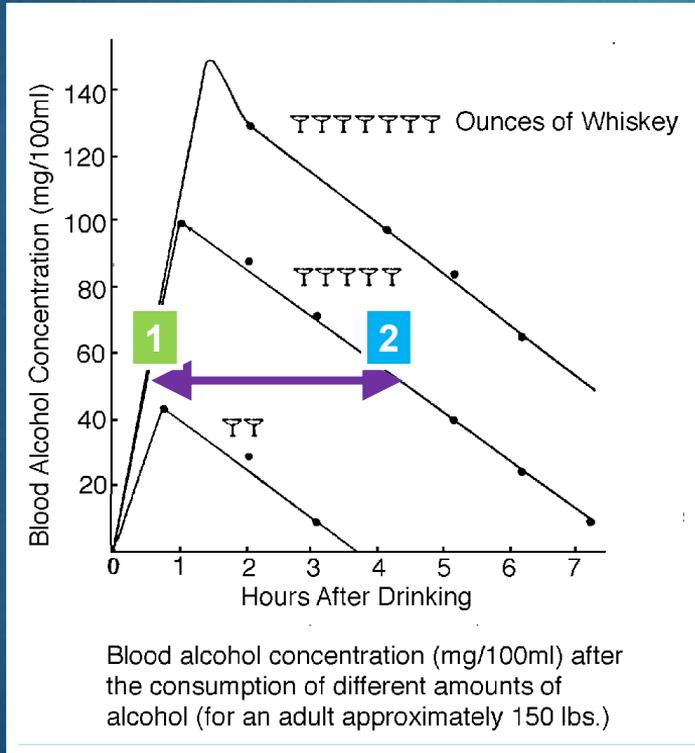
Anatabine

Ethanol (aka Alcohol)



- In chemistry, an alcohol is an organic compound in which a hydroxyl group (-OH) is bound to a carbon atom of an alkyl group.
- Most widely used depressant in the world

Blood Alcohol Content (BAC)



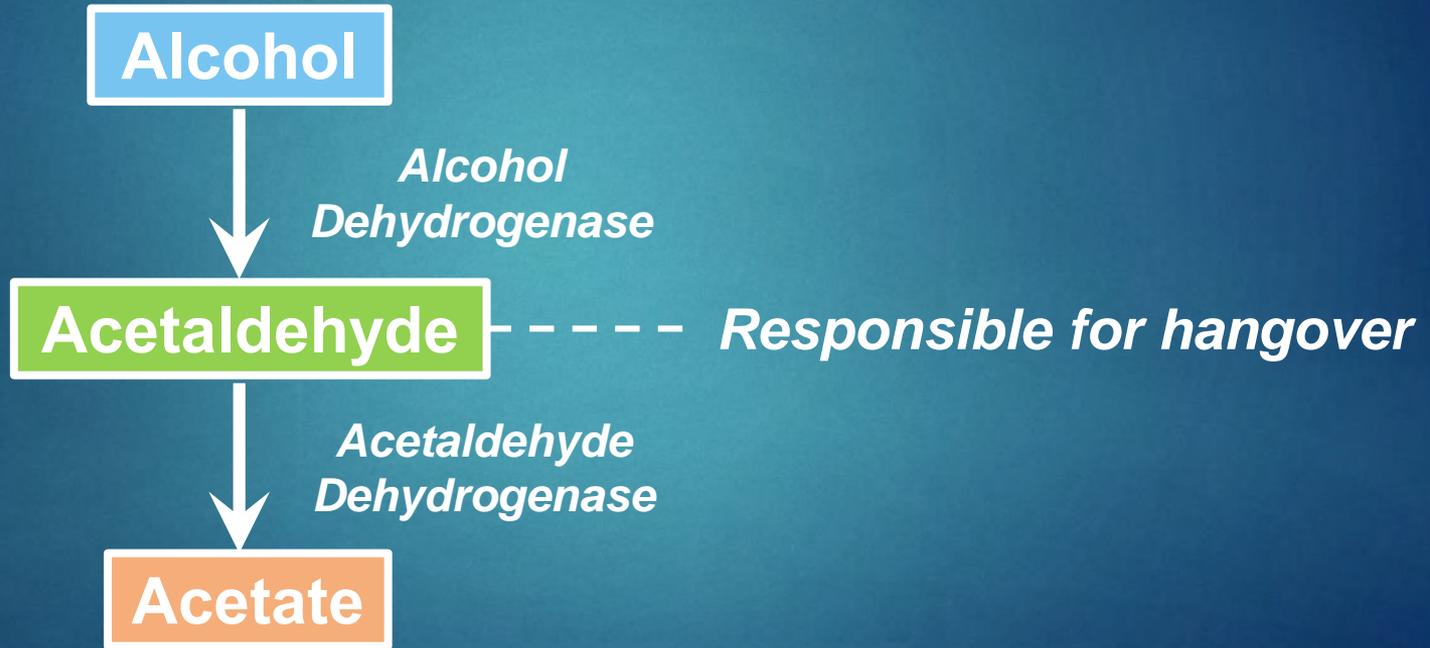
Similar BAC content
despite different time
points



Stimulant
Euphoria
Flushing

Depressant
Sedation
Fatigue

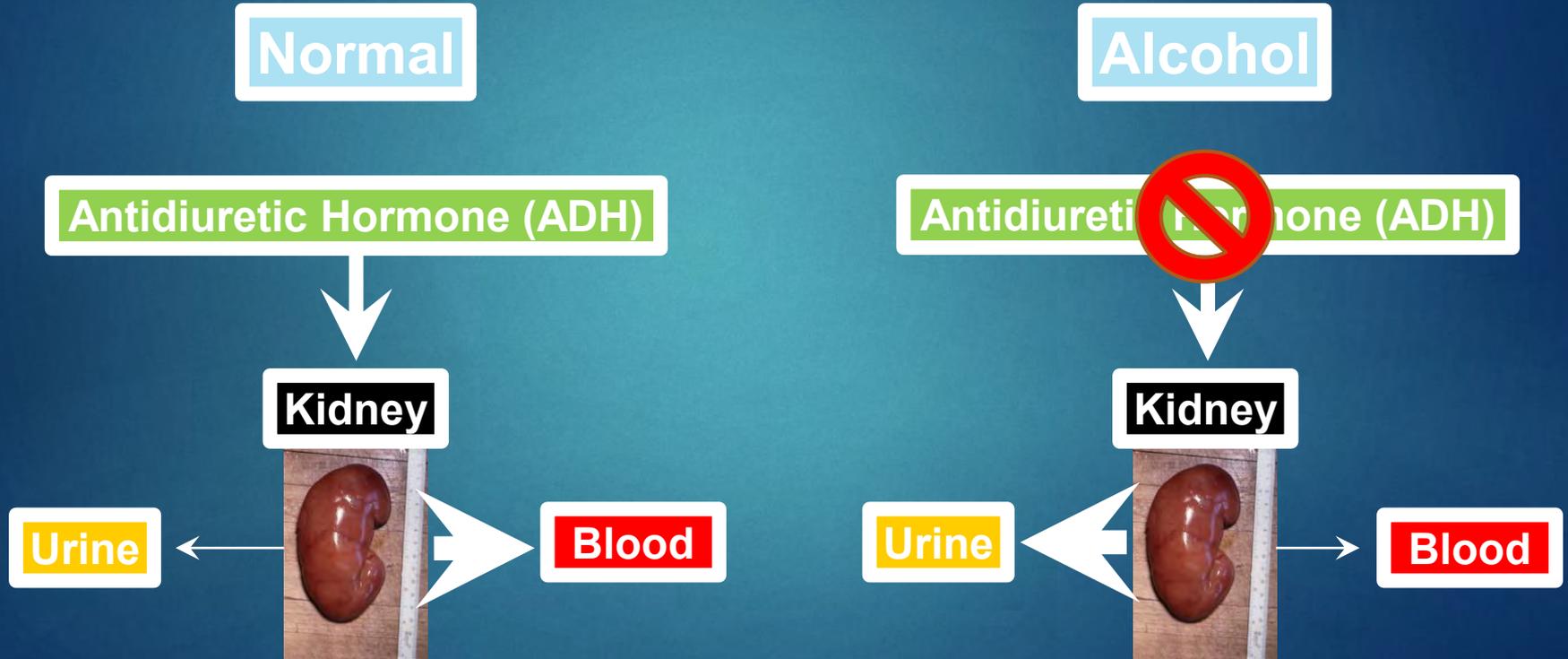
Alcohol Metabolism



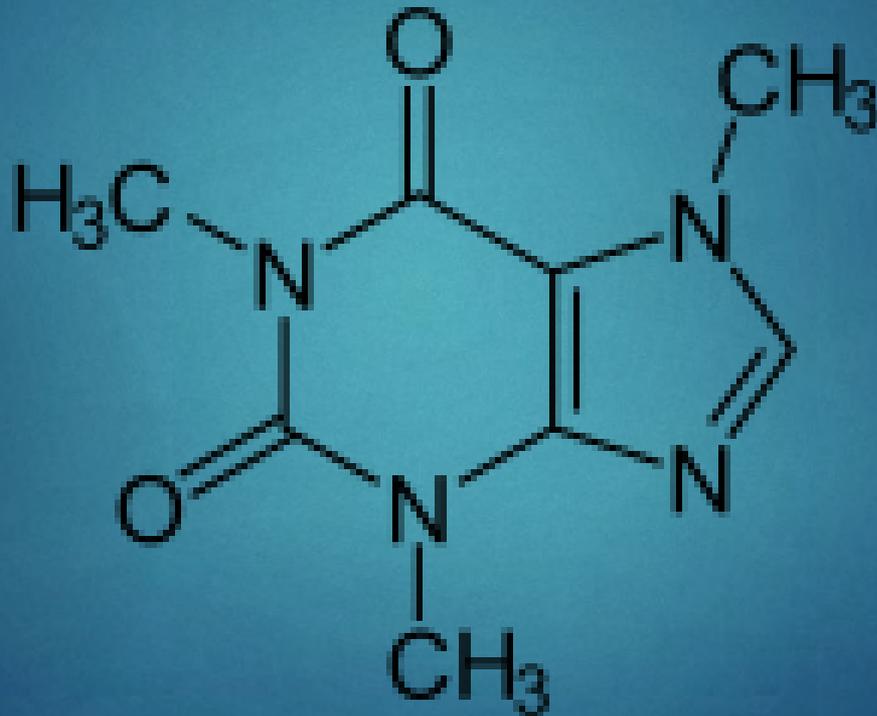
'Increasing the Hangover' is the way by which one FDA-approved medication effectively treats alcoholics



Why does alcohol consumption cause dehydration?



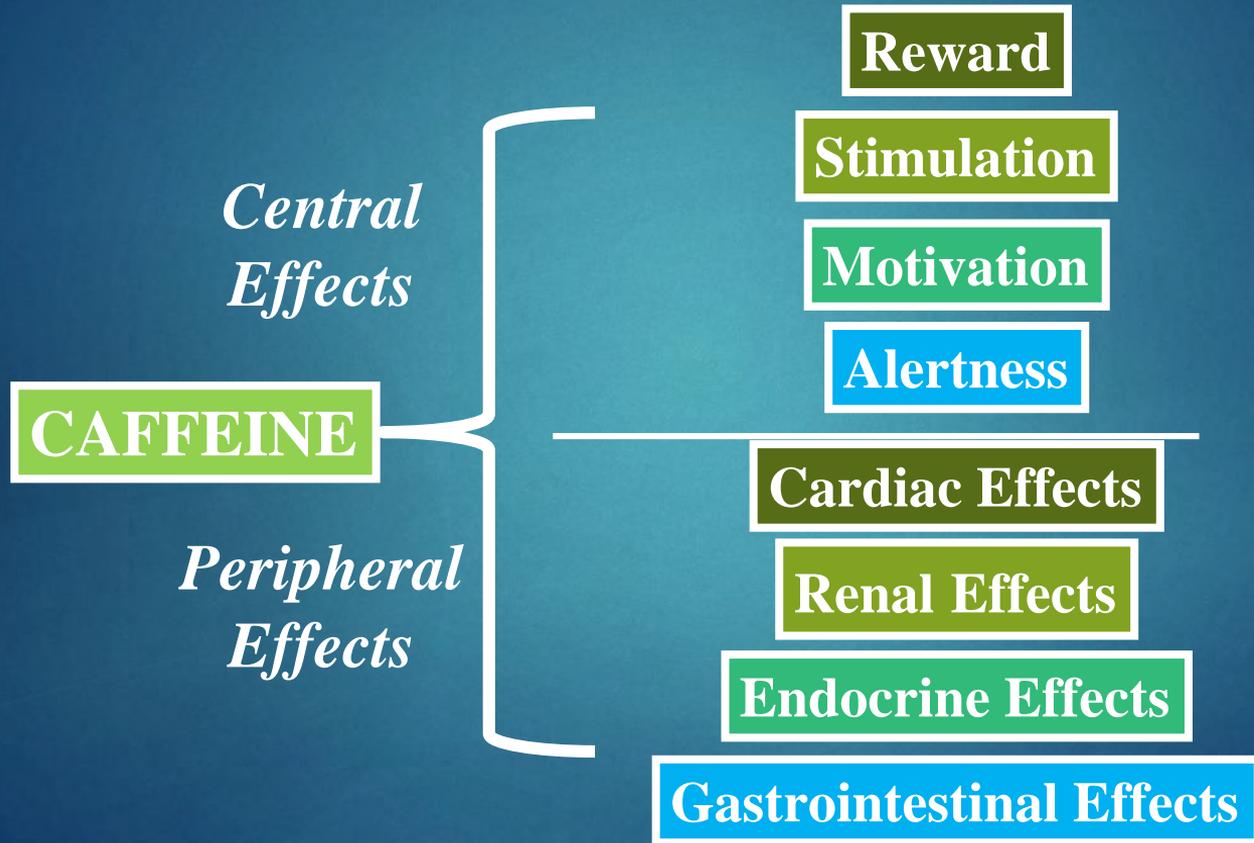
Caffeine



Caffeine Content in Coffee

Type of coffee	Size*	Caffeine**
Espresso, restaurant-style	1 oz. (30 mL)	40-75 mg
Espresso, restaurant-style, decaffeinated	1 oz. (30 mL)	0-15 mg
Generic brewed	8 oz. (240 mL)	95-200 mg
<i>Generic brewed, decaffeinated</i>	<i>8 oz. (240 mL)</i>	<i>2-12 mg</i>
Generic instant	8 oz. (240 mL)	27-173 mg
Generic instant, decaffeinated	8 oz. (240 mL)	2-12 mg
McDonald's brewed	16 oz. (480 mL)	100 mg
McDonald's Mocha Frappe	16 oz. (480 mL)	125 mg
Starbucks Latte	16 oz. (480 mL)	150 mg
<i>Starbucks Pike Place brewed</i>	<i>16 oz. (480 mL)</i>	<i>330 mg</i>

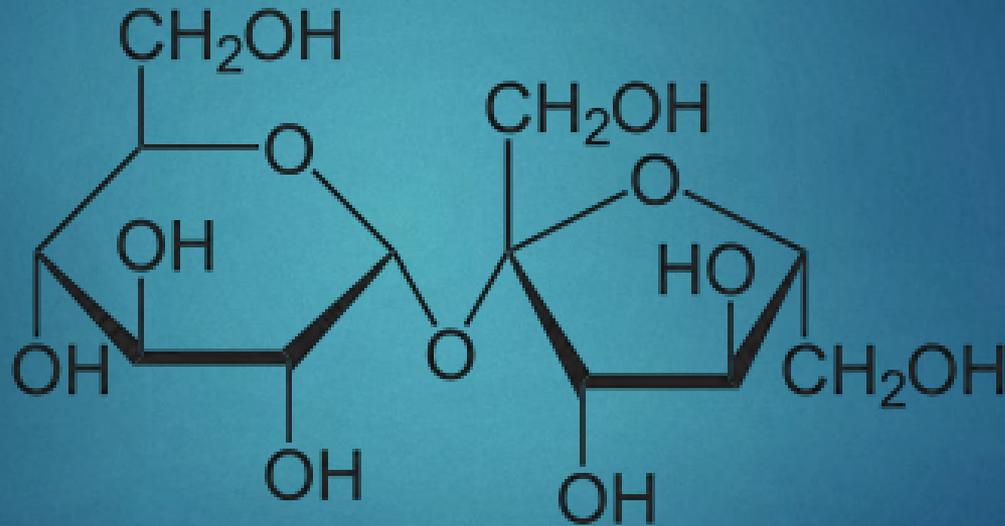
Caffeine Pharmacological Effects



Caffeine Physical Dependence and Withdrawal



Sucrose (Table Sugar)



Scientific Method Review

□ 1. Question

- What is it you are trying to find out?

□ 2. Hypothesis

- What do you think the answer will be? (*Can be done in an "if...then...because" manner*)

□ 3. Methods

- How will you test your hypothesis? What materials will you need? What methods will you use?

□ 4. Results

- What did your experiment tell you?

□ 5. Conclusion

- How can you apply the results of your experiment to a broader context? What have you learned? Direction for the future?