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Teacher Professional Development Materials for the Parkinson's Virtual Lab

Student Objectives
Parkinson's Virtual Lab

Students will be able to:

- list symptoms of Parkinson's Disease (PKD)

- explain the connection between the transmitter dopamine (DA) and PKD symptoms

- identify the two areas of the brain where DA neurons originate (begin) and terminate (end)

- describe how a DA neuron is different from a classical neuron

- explain vesicles' role in an action potential

- describe the phenomenon of DA neuron loss when PKD patients show symptoms

- explain Zigmond's Compensatory "Increased Release Model" And Garris' s Compensatory "Passive Stabilization Model".

- explain how anatomical regions of the rat's brain are located for surgery.

- interpret dopamine data, to determine whether neurons release more neurotransmitter with each action potential

- compare and contrast the two Compensatory models: Zigmond's, Increased Release Model and Garris' Passive Stabilization Model.

- describe how the bucket analogy explains the Passive Stabilization Model

Benefits
Parkinson's Virtual Lab

The Virtual Parkinson's Lab brings cutting edge research science into the classroom. Unlike reading from a textbook, students will be able to role-play a neurobiologist who conducts rat research in search for answers to the socially relevant issue of Parkinson's Disease (PKD). Virtual lab participants learn what is known about the connection of dopamine levels in preclinical stages of this disease, and then, students get to conduct virtual rat brain surgery; first to induce PKD-like conditions in the rats, and later to take electrical and chemical readings of what is happening in and around dopamine neurons. Students are testing a well-known theory; they collect data, and then interpret that data to see if it supports their hypothesis.

In addition to role-playing and being able to collect data to interpret, this virtual lab, allows students to see how the research process works, on a bigger, grander scale. Students learn that there is a cellular phenomenon that scientists do not understand. Data show that Parkinson's patients have normal neurologic function with up to 80% dopamine neuron loss. Data also show that although the number of neurons is greatly reduced, the overall amount of dopamine remains high. So the phenomenon that scientists are trying to explain, is how it is that cells compensate for this loss. Therefore, student in this virtual lab will test a commonly accepted theory, that the remaining neurons release more DA to compensate for the loss of the neighboring cells. As an additional theory is brought up later in the lab, students may get a sense of the connection between technology (instruments used to collect scientific data) and science. The Virtual Parkinson's lab models the important combination of scientists sharing their research with the improvement of technology that allows better and more accurate data to be collected, further improving explanations for how and why things happen.

The Virtual Parkinson's lab, as no other resource, demonstrates--what is happening at a cellular level in varying preclinical stages of Parkinson's patients. The virtual lab allows students to view animated models, *never before seen,* to help visualize what is happening in and among dopamine neurons the years and months leading up to when patients first start showing symptoms of the disease. One animated model helps students to visualize the cellular space in a specific part of the brain, where the neurotransmitter dopamine is being released and is used to help visualize what the theory might "look like" at the cellular level. And another animated graphic shows a mathematical model, so the neurologic data can be shown in real time.

Implementation Suggestions

The Virtual Parkinson's Disease Lab could be integrated into a number of different courses—from high school biology or health to undergraduate neurobiology courses. Depending on your curricular goals, you may choose to use the lab to focus students on specific areas to complement what they are learning. In addition to the general topics list below, review the student objectives and the science standards provided.

- Nervous System

- How the structure and function of a cell is related to its function

- How vesicles are involved in neuron communication

- Connection between engineering of instruments to the study of diseases

- How cells chemically communicate

- Connection of cell function with disease

- Conducting a scientific experiment (at the cellular level)

- Animal Research

These Standards are based on a draft of the "Next Generation Science Standards" made available in May 2012. Available http://nextgenscience.org/next-generation-science-standards.

MS.LS-SFIP: Structure, Function, and Information Processing
   a. Investigate and present evidence that the structure of cells in both unicellular and multicellular organisms is related to how cells function.
   c. Construct an explanation for the function of specific parts of the cell including: nucleus, chloroplasts, and mitochondria and the structure of the cell membrane and cell wall for maintaining a stable internal environment.

HS.LS-SFIP: Structure, Function, and Information Processing
   a. Obtain and communicate information explaining how the structure and function of systems of specialized cell within an organism help them perform the essential functions of life.
   e. Use evidence to support explanations for the relationship between a region of the brain and the primary function of that region.
   f. Gather and communicate information to explain the integrated functioning of all parts of the brain for successful interpretation of inputs and generation of behaviors.

MS.ETS-ETSS: Links Among Engineering, Technology, Science, and Society
   a. Provide examples to explain how advances in engineering have resulted in new tools and instruments for measurement, exploration, modeling, and computation that enable new scientific discoveries, which in turn lead to the development of entire industries and engineered systems.

Teacher's Lab Guide
## Parkinson's Virtual Lab

Lab Overview

The Virtual Parkinson's Virtual Lab is divided into several parts.

Part 1: Tutorials

There are a total of five (5) tutorials in which students will learn the background necessary to get the most out of the virtual lab. Students read text and interact with animated graphics in order to learn about the connection of dopamine and Parkinson's Disease (PKD). At the end of every tutorial there is quiz. You may consider using the student handouts that students can complete to help focus them through these reading sections.

1st Tutorial: Introduction to Parkinson's
2nd Tutorial: Introduction to Dopamine Neurons
3rd Tutorial: Compensation of Dopamine Neurons
4th Tutorial: Bringing It All Together
5th Tutorial: Designing Your Experiment
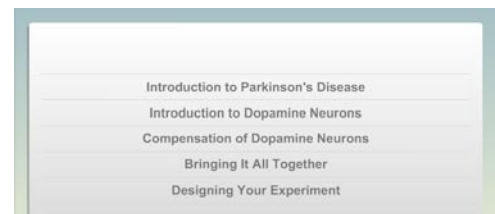
Part 2: Lab Work to Lesioning Surgery

Students anesthetize their virtual rat, use a rat atlas to determine the location of the substantia nigra, and then insert a needle to lesion neurons in order to simulate PKD neurologic degeneration.

Part 3: Surgery to Implant the Voltammetry System

Students drill holes for the recording electrode, reference electrode, and stimulating electrode. This will allow students to collect data on the amount of DA molecules in this (striatum) area of the brain.
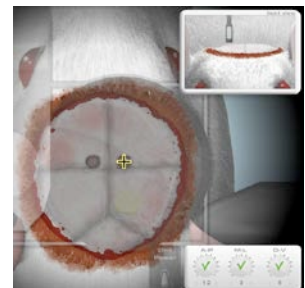
Part 4: Lab Results
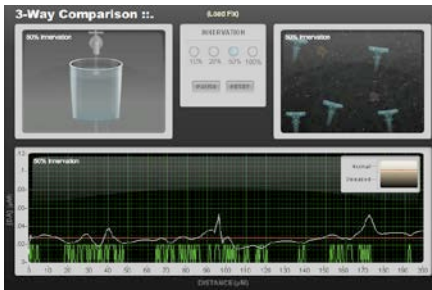
Students interpret data by viewing graphs and computer models. Students will determine whether or not the hypothesis was supported by the data. The well-established explanation for the 80-20 phenomenon that was tested in the experiment is compared to another new theory called Passive Stabilization Model.

Part 5: Assessment: Word Drag

Students solidify what they have learned by completing a 4 paragraph summary of their virtual lab experience. This assessment can be interactively online, or students can complete this assessment on paper (provided in the student handout).

Teacher Guide to Part 1

Summary of the Tutorials

The first part of this lab is where students have background reading about Parkinson's Disease, dopamine's role in PKD, and an explanation what hypothesis they will be testing in this virtual lab. The Mind Project has developed student handouts that will help focus students on what they need to know to perform the lab procedures later in their virtual experience.

Here are the big picture ideas, that students should walk away with after each tutorial section.

1st Tutorial Summary: Parkinson's Disease is associated with a significant decrease in the overall amount of dopamine tone in certain parts of the brain. Parkinson's patients suffer symptoms such as resting tremors, rigidity, slow movement, or no movement.

2nd Tutorial Summary: Dopamine (DA) neurons begin in the substantia nigra and end in the striatum. The DA neurons differ from classical neurons in that they have transporters and receptors located outside the synaptic cleft. This means that the neurotransmitter DA floats not just between synaptic clefts, but also in the extracellular space. This is called volume transmission and creates a baseline of DA in these areas of the brain that we call dopamine tone.

3rd Tutorial Summary: The phenomenon of PKD, is that patients show NO symptoms until they have lost 80% of their DA neurons. Meaning, somehow the brain is able to compensate for the DA

that is lost. Dopamine Tone is maintained until only 20% of the neurons remain. This virtual lab was designed to explore two explanations. The first is the Increased Release Compensatory Model, which says the remaining DA neurons are able to increase the number of DA neurotransmitters released with each action potential. This is the hypothesis that will be tested in the virtual lab.

4th Tutorial Summary: Since the DA neurons' cell bodies are located in the substantia nigra, this is where researchers can lesion (poison) cells to emulate PKD conditions in rats.

5th Tutorial Summary: The hypothesis being tested in this virtual lab is that "Increased DA release maintains DA tone during the presymptomatic phase of PKD." This will be tested by measuring DA release in a rat with PKD. The procedure for this is to kill DA neurons in the brain, and then use microsensors (via a voltammetry system) to measure DA release and tone.

Tutorial Answer Keys

Tutorial 1 Quiz: Introduction to Parkinson's Disease

1. What is Parkinson's Disease
    A. Pulmonary disorder
    B. *Movement disorder
    C. Cognitive disorder
    D. Cardiovascular disorder
2. Which is NOT a primary symptom of Parkinson's Disease?
    A. Resting tremor
    B. Rigidity
    C. * Hyperactivity
    D. Slow or no movement
3. Which is the cause of most cases of Parkinson's Disease?
    A. Genetic
    B. Bacteria
    C. Environmental
    D. *Unknown
4. How is the brain damaged in Parkinson's Disease?
    A. Widespread loss of neurons
    B. Shrinkage
    C. General loss of tissue
    D. *Loss of dopamine neurons in the substantia nigra and striatum

5. Which is the group of people who typically get Parkinson's Disease?
    A. *Elderly
    B. Men
    C. Women
    D. Children
6. How is Parkinson's Disease treated?
    A. Dietary changes
    B. Hypnosis
    C. *Drugs and neurosurgery
    D. No treatment currently exists

Tutorial 2 Quiz: Introduction to Dopamine Neurons

1. Where are dopamine neurons associated with Parkinson's Disease located in the brain?
    A. *Originate in the substantia nigra and terminate in the striatum
    B. Terminate in substantia nigra and originate in the striatum
    C. Originate in both the substantia nigra and striatum
    D. Terminate in both the substantia nigra and striatum
2. Which is NOT a primary structure of the dopamine neuron?
    A. Dendrite
    B. *Reticulum
    C. Cell body
    D. Axon
3. The symptoms of Parkinson's Disease are the result of reduced levels of ____ in the brain.
    A. *Dopamine
    B. Serotonin
    C. Adrenaline
    D. Tyrosine
4. Which mechanisms do NOT regulate dopamine in extracellular space?
    A. Release
    B. Uptake
    C. *Synthesis
    D. Diffusion

5. How does dopamine typically control another neuron?
    A. *Affecting postsynaptic receptors via volume transmission
    B. Affecting presynaptic receptors via volume transmission
    C. Affecting postsynaptic receptors via synaptic transmission
    D. Affecting presynaptic receptors via synaptic transmission

Tutorial 3 Quiz: Compensation of Dopamine Neurons

1. What is dopamine tone?
    A. *Constant level of extracellular dopamine
    B. Surges of extracellular dopamine
    C. Transient changes of extracellular dopamine
    D. Vesicular dopamine stores

2. Symptoms of Parkinson's Disease emerge when dopamine terminal loss exceeds____.
    A. 10%
    B. 20%
    C. *80%
    D. 100%

3. Why do symptoms of Parkinson's Disease emerge?
    A. Dopamine tone increases
    B. *Dopamine tone decreases
    C. Postsynaptic neurons die
    D. Postsynaptic neurons proliferate

4. What is the primary compensatory response during the presymptomatic phase of Parkinson's Disease?
    A. *Maintenance of dopamine tone
    B. Maintenance of postsynaptic neurons number
    C. Maintenance of dopamine neuron number
    D. Maintenance of brain size

5. According to Zigmond's Increased Release Theory, how is dopamine tone maintained during the presymptomatic phase of Parkinson's Disease?
    A. Compensatory increase in the number of postsynaptic neurons
    B. Compensatory increase in the number of dopamine neurons
    C. Compensatory decrease in dopamine release and diffusion
    D. *Compensatory increase in dopamine release and diffusion

Tutorial 4 Quiz: Bringing It All Together

1. In what parts of the brain do DA neurons originate and terminate?
    A. Thalamus and Global Palidus
    B. Substantia Nigra and Subthalamic Nucelus
    C. Subamalic Loop
    D. * Substantia Nigra and the Striatum

2. What part of the brain is responsible for communicating movement signals between the brain and the body?
    A. Thalamus
    B. Substantia nigra
    C. *Motor cortex
    D. Lateral ventricle

3. What part of the brain sends "Go" and "Stop" signals to the Motor Cortex?
    A. *Thalamus
    B. Substantia nigra
    C. Striatum
    D. Lateral ventricle

4. All of the following are true about how Parkinson's patients maintain normal motor function EXCEPT:
    A. DA tone in the striatum is high
    B. Motor Cortex is signaled by the Thalamus
    C. *DA tone in the Substantia Nigra is high
    D. The Basal Ganglia modifies information and sends it to the Motor Cortex

5. Parkinson's patient's may show tremors once...
    A. 20% of DA neurons have died
    B. *The motor neurons receive significantly fewer messages
    C. The neurons in the motor cortex are over stimulated
    D. The DA tone in the substantia nigra is low

6. In Parkinson's Disease, the loss of dopamine ____?
    A. *Inhibits the thalamus and cortex
    B. Activates the thalamus and cortex
    C. Inhibits the cortex and activates the thalamus
    D. Activates the cortex and inhibits the thalamus

Tutorial 5 Quiz: Designing Your Experiment

1. The hypothesis of the experiment is that Dopamine Release "_____" during the preclinical phase of Parkinson's Disease.
   - A. Increases
   - B. Evaporates
   - C. *Does not increase
   - D. Burns

2. The design of the experiment is "_____" in an animal model of Parkinson's Disease.
   - A. *Measuring dopamine release
   - B. Measuring dopamine synthesis
   - C. Measuring new dopamine neurons
   - D. Measuring new dopamine synapses

3. What is the animal of Parkinson's Disease used in the experiment?
   - A. Monkey
   - B. *Rat
   - C. Sloth
   - D. Toad

4. If we are trying to kill DA neurons with a toxin, we will want to kill the cell bodies of the neurons. Therefore, what part of the brain should we inject the neurotoxin?
   - A. *Substantia Nigra
   - B. Corpus
   - C. Cortex
   - D. Thalaums

5. The procedure of the experiment is, "____"in a dopamine-lesioned rat model of Parkinson's disease.
   - A. CT scan measurements of dopamine release
   - B. PET scan measurements of dopamine release
   - C. *Microsensor measurements of dopamine release
   - D. X-ray measurements of dopamine release

Lab Work to Lesioning Surgery

The big idea: purpose of this first rat surgery is to lesion (kill) DA neurons in the rat's substantia nigra to induce Parkinson's disease.

Dressing for Surgery

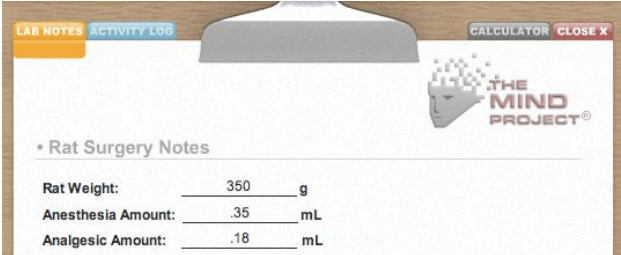Students put on lab coat, gloves, and mask.

Anesthetizing Rat for Surgery

Students will be preparing the rat for surgery, weighing, and giving the rat a shot of anesthesia and analgesic.

> Their virtual rat will weigh <u>350 grams</u>
>
> To calculate anesthesia <u>350/1000 = .35 mL</u>
>
> To calculate analgesic = <u>.35 mL/2 = .18 mL</u>

Students get these equations either in the virtual lab (in the ERM) or in the student handout. As part of the virtual experience, student must enter these values on their virtual clipboard, <u>and hit save</u>. This ensures that they make the correct calculations because they must enter the correct numbers.

Lesioning Prep and Surgery

You may want to emphasize that rats used in research are well cared for, and all research has gone through scrupulous board review before being implemented.

Protocol at this station includes:

1. Shave the rat's head

2. Apply iodine to rat's scalp

3. Apply lubricant to both eyes

4. Make incision to rat's scalp

5. Place two clamps on the skin to expose the skull
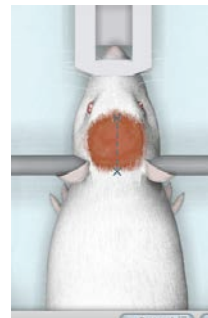
FYI: A Stereotaxic Machine is a device that allows researchers to precisely align items like drills, needles, and electrodes to certain areas of the rat's skull and brain. The researcher can then record those precise coordinates for later use, or review coordinates previously documented by others to use as starting points.

6. Record Bregma Coordinate

Depending on how much emphasis you want to place on this, we have developed "Brain Coordinates & Reading a Rat Atlas" handout to help students learn about the coordinate system and tips on how to read an atlas. Within the lab, once students review the rat atlas pages, their clipboard will have the coordinates already there. Students will dial these coordinates using the stereotaxic dials.
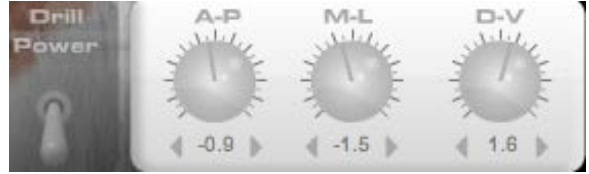
	A-P	M-L	D-V
Bregma	0	0	0
Lambda	-9	0	0
Substantia Nigra	-5.6	2	-7.6

7. Record Lambda Coordinates

8. Record Substania Nigra Coordinates

9. Align drill bit over substantia nigra.

10. Turn on and lower drill (the front view shows the drill at eye level, and students should see it turn) Student will not drill to the D-V depth of the substantia nigra, they are just drilling a hole in the skull above the anatomical position.
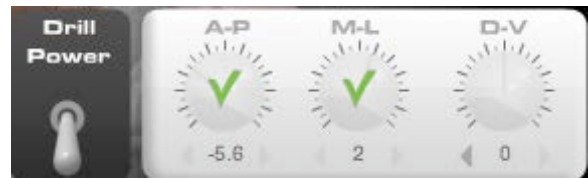
The drill is removed and a needle is attached to the stereotaxic arm.

11. Align the needle over the substantia nigra and lower it to the proper depth.

12. Program the syringe pump (to deliver the neurotoxin through the needle) Program the syringe pump for 2 ul @ 10 min.

13. Raise the needle

This portion of the experiment is complete, so you must now mend the rat by filling the hole in the skull and suturing the skin back to its original formation.

14. Apply bone wax & staple the incision.

The rat will now spend 2 weeks recovering from the procedure. This will allow for both the rat to heal, and for the lesioning procedure you just performed to take full effect.

Surgery to Implant the Voltammetry System

Now that the rat has healed, it's time to perform a Voltammetry procedure. This involves implanting various types of electrodes in the brain to measure certain brain activities.

15. Cut away circle of skin to reveal the skull

16. Align drill with **Reference** electrode coordinates

17. Drill hole in skull (just -1) to give access to the brain

18. Align drill with **Recording** electrode coordinates

19. Drill hole in skull (just -1) to give access to the brain

20. Align drill with **Stimulating** electrode coordinates

21. Drill hole in skull (just -1) to give access to the brain
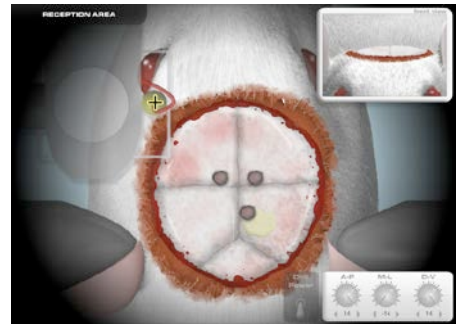
	A-P	M-L	D-V
Substantia Nigra	-5.6	2	-7.6
Reference Electrode	1.2	-3	-4
Recording Electrode	1.2	3	-4.5
Stimulating Electrode	-4.6	1.6	-8

This time, the stereotaxic drill arm will be exchanged for an arm that is used to implant three electrodes in the rat's brain.

22. Insert the Reference electrode

23. Insert the Recording Electrode

24. Insert the Stimulating Electrode
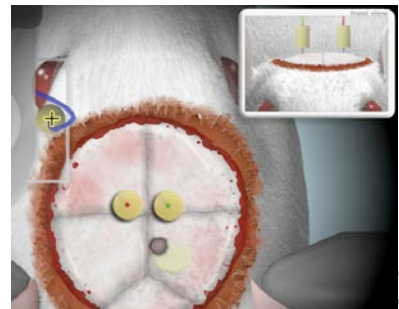
Note to Teacher: the large image in the screen is looking from above, and the smaller image in the upper right-hand corner is looking at the rat at eye level. This should help students with the perspective that they are lowering drills, needles, and electrodes into the brain.

Once all the electrodes are in place, data can be collected.

Teacher Guide to Part 4

Lab Results

The lab results are given to students in the form of graphs. While raw data is not included in the virtual lab, reiterate to students that this virtual lab is based on real data gathered in Dr. Garris' labs. The data show that amount of dopamine released decreases proportionally with the percent of denervation (death of neurons).
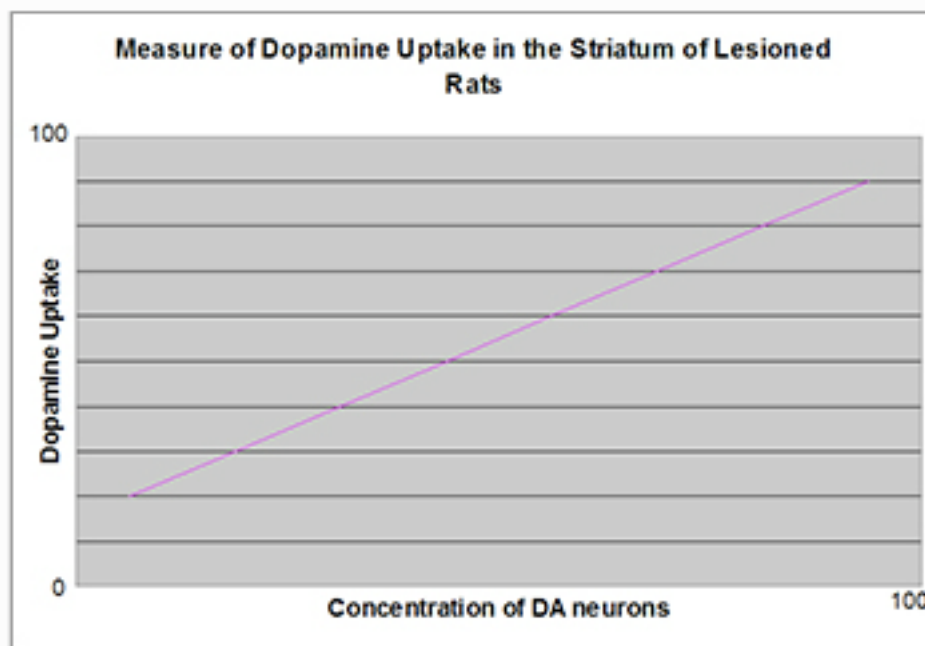
The Hypothesis we tested was that dopamine release does not increase to maintain dopamine tone during the preclinical phase of PKD. The prediction was that DA tone will drop with the loss of dopamine neurons.

The data support our prediction. DA neurons in fact, are NOT releasing more DA to compensate for the reduced number of neighboring DA neurons. Therefore, our data do NOT support the Increased Release Model, and dopamine tone must be compensated another way.

Which leads us into a discussion of another model to explain how the body compensates for the loss of DA neurons. It is called the Passive Stabilization Model.

The questions in the student handout require them to construct a graph of the data and then to come up with their conclusions. While the handout helps students focus on what they should be learning, it's the interactive graphic that will really drive the point home. Encourage them to "play" with the various percentages, and to take note of what is happening with the DA that is diffusing among the cells.

Number 11 in the student handout includes a table where students compare and contrast the two compensatory models. They should be isolating what makes them different, but also on what point the theories agree.

Using mathematical models, animated graphics, and concrete analogies to help explain theories may be a new to your students. In the latter parts of this virtual lab, the data collected in the experiment is displayed within a computer program, called the Passive Stabilization Program. This enables to show in an animated way, what is happening on a cellular level. At first students may not be greatly impressed with the bouncing white line (nerve impulses), and the changes in the green lines (location of functioning neurons), but the fact that scientists can collect measurements on this small of a scale is worthy of awe! The animated graphic is a representation of a real mathematical model that was developed by Dr. Paul Garris. In addition, the Passive Stabilization Model is explained using a concrete analogy of a Bucket with water in it. Dr. Garris developed this analogy to help individuals better understand his explanation of what is happening in between these dopamine neurons.

Teacher Guide to Part 5

Assessment (Word Drag)

This is an interactive fill in the blank activity that results in a four-paragraph overview of the entire virtual lab. You may choose to have students show you that they completed it (they can't move on in the lab until they've done it completely). We do provide a paper version that is available in the student handouts.

Parkinson's disease is a **Degenerative** , neurological disorder of unknown causes. Early symptoms include a **Resting Tremor** (trembling of the hand while it's at rest) and **Rigidity** (muscle stiffness). Parkinson's disease is considered a **Motor** disorder as it affects the movement of the body.

Patients with Parkinson's disease suffer from the loss of **Dopamine** neurons in the **Substantia Nigra** . Known as the nigrostriatal pathway, these neurons have their cell bodies in the substantia nigra. Their axons run through the **Mediastianatal Bundle** and end in the **Striatum** .

When dopaminergic neurons release dopamine, the dopamine molecules will **Diffuse** until they are taken up by a **Dopamine Transporter** or attach to a target cell. This is known as **Volume Transmission** .

Volume transmission results in a background concentration of dopamine, known as **Dopaminergic Tone** . This allows for signals to pass smoothly from the **Thalamus** to the **Motor Cortex** and then out to the rest of the body.

Parkinson's disease patients do not show visible symptoms until **80%** of the dopaminergic neurons in the striatum have died. Scientist don't know how dopaminergic tone is maintained until then. Dr. Zigmond has put forth the **Compensatory Release** model, which theorizes that the remaining dopaminergic neurons release **More** dopamine with each action potential.

Dr. Garris disagrees with this theory. He has proposed the **Passive Stabilization** model, which theorizes that dopaminergic tone is maintained because of the relationship between dopamine release and **Uptake** . As a patient loses dopamine neurons, he or she not only loses a source of dopamine **Release** , but also a source of dopamine uptake. Therefore, the net concentration of dopamine molecules remains the **Same** . This stabilization is disrupted as the **Glial** begin to compete with the dopaminergic neurons for the uptake of dopamine molecules.

Materials for Students

Parkinson's Vocabulary Sheets

Because this lab has a lot of vocabulary that may be new to students, we are providing a vocabulary handout, with the acronyms used throughout the lab, as well as other terminology. The first page is an empty handout that you may choose to have students fill in as they complete the lab. The answer key follows, but you may choose to give the key to students to speed up their understanding while working within the virtual lab.

Parkinson's Student Handouts

To help focus your students, there are pdf files that you can print out as handouts that your students can complete as the move through the virtual lab. The answer key to those handouts are below.

Name _____ Class _____ Date _____

Virtual Parkinson's Disease Lab: Vocabulary Sheet

Compensatory Model (theory): the idea that something within the dopamine system is able to compensate for a decrease number of DA neurons that is able to keep the overall DA tone relatively high even with significant neuron loss. Two compensatory models are discussed in this virtual lab—Increased Release Model and the Passive Stabilization Model.

Dopamine (abbreviated DA): a specialized neurotransmitter released by DA neurons. Low level of DA has been found associated with Parkinson's Disease symptoms.

Dopamine Transporters (abbreviated DAT): Receptors on the surface of DA neurons that are called "uptake sites" because they are responsible for absorbing DA back into the presynaptic neuron. The DAT can be located in the synaptic cleft (like other neurons) but are also found outside of the synaptic cleft.

Denervation: The number of neurons that have died and are no longer functioning

Dopamine Tone: (sometimes called dopaminergic tone): Is the concentration of DA molecules that exist in among and between cells in a certain part of the brain – like the striatum. DA tone can be measured using microelectrodes placed in the region of the brain being studied.

Electrodes: in our virtual experiment we use chemical microsensors to record the amount of DA release and electrical conductors to stimulate cells to fire.

Innervation: The number of remaining (living), functioning neurons.

Lesioning: Placing a neurotoxin (poison) in parts of the brain to kill neurons and to simulate a disease. In the case of this virtual lab, the neurotoxin is placed in the substantia nigra (where dopamine neurons originate) to induce Parkinson's Disease symptoms in rats.

Parkinson's Disease (abbreviated PKD): A disease caused by the loss of the neurotransmitter, dopamine. Symptoms of the disease include resting tremor, and rigidity. There is no cure, but symptoms can be treated.

Presymptomatic: (or preclinical) before a patient shows symptoms (symptoms for PK patients include resting tremors or rigidity)

Uptake: the process of taking up neurotransmitters into the neuron to be used again.

Voltametry System: The entire electrochemical technique that is used to monitor release and uptake of DA. This includes implanting electrodes into the brain, and sending data back to a computer where it can be analyzed.
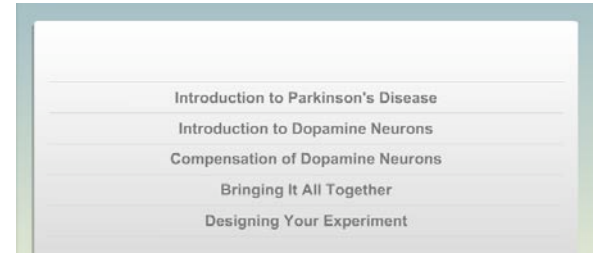
Volume Transmission: The movement of molecules out of the synaptic cleft allows DA terminals to provide DA molecules to any surrounding cells, not just one.

Teacher's Answer Key...

<div align="center">

The Mind Project's
Parkinson's Disease Virtual Lab: Part 1

</div>

First Tutorial: Introduction to Parkinson's Disease

1. What are the primary symptoms of Parkinson's Disease (PKD)?

   Resting tremor, rigidity, slow movement (bradykinesia) or no movement (akinsia) and postural instability.

2. What happens in the brain to cause the symptoms of PKD?

   The brain (specifically the substantia nigra and striatum) loses the neurotransmitter dopamine.

3. While PKD can't be cured, what treatments do patients usually receive to manage their symptoms?

   Drugs that increase brain dopamine, or mimic dopamine, or deep brain stimulation surgery.

Second Tutorial: Introduction to Dopamine Neurons

4. Where are dopamine neurons in the brain?

   Start in the substantia nigra and end in the striatum

5. What is the structure of a dopamine neuron?

   Axon terminal, dendrite, cell body, axon, DAT, vesicles, & synapse

Launch the "Transmission of Dopamine" animation to learn about how dopamine neurons communicate.

6. How are dopamine neurons different than "classical" neurons?

   Dopamine uptake sites (Dopamine Transporters=DAT) and receptors are located outside the synaptic cleft, not inside like with classical neurons.

7. What role do vesicles play in the dopamine neurotransmitter being released?

Vesicles hold dopamine at the terminal membrane until an action potential causes Exocytosis—which is when the vesicle fuses with the membrane to release dopamine into the synaptic cleft.

8. What two places might a free-floating DA molecule end up?

Being taken up by a DAT (to be recycled within a new vesicle), or attaching to a target cell receptor, where binding produces a chemical change to induce an action potential in the post synaptic neuron.

9. What is volume transmission?

The movement of molecules out of the synaptic cleft allows DA terminals to provide DA molecules to any surrounding cells, not just one!

10. What is dopamine tone?

The baseline concentration of DA in between cells in the brain (esp. striatum).

Third Tutorial: Compensation of Dopamine Neurons

11. Fill in the blank.

The phenomenon scientists are trying to figure out is how the body is able to compensate for ____(80%)____% neuron loss , while maintaining high overall dopamine tone. Patients only begin to show symptoms when the percentage of remaining neurons falls below ____(20%)____%.

Launch the "Compensation of Dopamine Neurons" animated graphic and answer the following questions.

Refer to these definitions to help you understand the compensation model

Nerve innervation (inervated regions): the functioning nerves that appropriately connect and produce normal body functions

Denervated: death of the nerve

12. Zigmond's explanation of how dopamine tone is maintained in his *Increased Release Compensatory Model*. After watching the graphic, explain this theory, watching carefully the amount of DA released at each nerve ending.

DA neurons must release more DA neurotransmitters with each action potential to compensate for the lost neurons. The additional neurotransmitters diffuse to areas and bind to receptors on functioning neurons (innervated regions).

Fourth Tutorial: Bringing It All Together

13. In what part of the brain are the cell bodies of DA neurons?

    In the substantia nigra

14. In what part of the brain do DA neurons end (terminate) and release DA neurotransmitters?

    In the striatum

15. How does the motor cortex influence the body? What role does the basal ganglia play in the process?

    The motor cortex is the part of the brain that delivers movement signals to the body. Information from the motor cortex is sent to the basal ganglia, is modified, sent to the thalamus and then back to the motor cortex.

16. In Parkinson's Disease, the loss of dopamine inhibits what parts of the brain?

    Thalamus and cortex

17. What happens in the brain of a Parkinson's patient's that causes tremors?

Messages are not sent to the motor cortex, leading to fewer motor neurons being fired.

Fifth Tutorial: Designing Your Experiment

18. What is the hypothesis we will be testing in this virtual lab?

    Dopamine release does not increase to maintain dopamine tone during the preclinical phase of PKD. The prediction is that DA release will drop with the loss of dopamine neurons.

19. How will we test our hypothesis? (experimental design)

    We will measure dopamine tone, dopamine release, and dopamine uptake in a rat with artificially induced PKD.

20. What is our procedure?

    Insert a toxin (poison) to kill DA neurons in the brain, and then use a recording electrode to measure DA release and uptake. This requires us to implant electrodes into strategic areas of the brain.
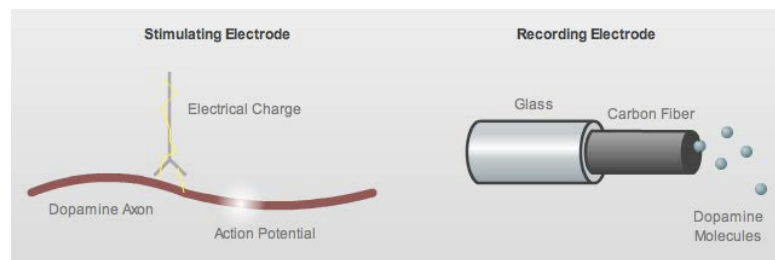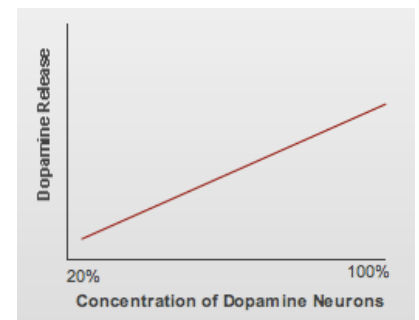
Answer Key…

The Mind Project's
Parkinson's Disease Virtual Lab: Parts 2-5
From The Mind Project's Virtual Parkinson's Lab

Prepping Rat and Performing Lesioning Surgery

Before you can begin surgery on your rat, you must weight, anesthetize your rat to put it to sleep, and also inject it with an analgesic to decrease pain after surgery.

| To calculate the amount of **anesthesia** needed	$\dfrac{\text{Weight of the rat (g)}}{1000} =$	mL
To calculate the amount of **analgesic** needed	$\dfrac{\text{Weight of the rat (g)}}{1000} / 2 =$	mL

Rat Surgery Notes

1. Rat Weight _____350_____ g

2. Anesthesia Amount _____.35_____ mL

3. Analgesic Amount _____.18_____ mL

In order to kill neurons in the substantia nigra, where dopamine neurons originate (start), we must use a Rat Atlas to determine the approximate coordinates. A-P = anterior/posterior; M/L = medial/lateral, and D-V = dorsal/ventral. The following is a completed version of the virtual clipboard you will find in the virtual lab.

	A-P	M-L	D-V
Bregma	0	0	0
Lambda	-9	0	0
Substantia Nigra	-5.6	2	-7.6

Surgery to Implant Electrodes in the Rat's Brain
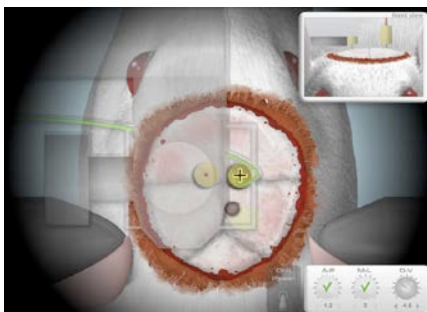
After our rat has recuperated for 2 weeks, and the neurotoxin has had time to take affect, we perform another surgery to implant electrodes that will allow us to collect data on how much dopamine is released. This system is called **Voltammetry**. In this surgery we need to drill additional holes

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in the skull for additional equipment. Three types of electrodes are listed below, along with the reason each is needed:

- **Recording electrode** = This electrode gathers the data we will be studying. We want to know how much DA is in the extracellular space in the striatum. Essentially we are measuring the amount of dopamine tone.
- **Stimulating electrode** = This electrode is inserted near the origin of the neurons (in the substantia nigra) so we can stimulate the neurons to fire, and therefore study the DA result of those firing neurons.
- **Reference electrode** = This electrode acts as the control

	A-P	M-L	D-V
Substantia Nigra	-5.6	2	-7.6
Reference Electrode	1.2	-3	-4
Recording Electrode	1.2	3	-4.5
Stimulating Electrode	-4.6	1.6	-8

Once you've drilled the holes for the additional electrodes, you'll insert the electrodes to the proper depth (D-V), then you'll detach the electrode and raise the sterotaxic arm.

The Big Idea: Notice that the stimulating electrode is placed very close to the location where we inserted the neurotoxin. The coordinates for the substantia nigra and the stimulating electrode are very close in proximity. Remember, we added the neurotoxin in order to kill the cell bodies of dopamine neurons within the substantia nigra so we can replicate on a cellular level what is going on in Parkinson's patients.

Lab Results

Reading the Dopamine Data from the Rat's Brain

4. Looking Back: What is the hypothesis we tested?

    Dopamine release does not increase to maintain dopamine tone during the preclinical phase of PKD.

Lab Results

5. What are we looking to compare in our data?

    We are comparing the percent of remaining neurons with how much DA is released when those neurons are stimulated.

6. What other data are we looking at?

    Amount of DA taken up by the DA transporter molecules.

7. Graph Title:_____

<table>
<tr>
<td>

</td>
<td>
Use the space on the left, create a graph with the data from our experiment. Be sure to include a:

        a) title for the graph

        b) label for the y axis

        c) label for the x axis

        d) line that represents the data
</td>
</tr>
</table>

8. Do the data support our hypothesis? How do you know?

> Yes, the data supports our hypothesis, because dopamine release and dopamine uptake both decrease as neurons die off.

Passive Stabilization Compensatory Model

9. What role does a glial cell have in the Passive Stabilization Compensatory Model?

> It takes up DA. When the number of DA neurons releasing DA decreases, the uptake of glial cells are more noticeable.

10. What role does the concept of "Uptake" play in the Passive Stabilization Compensatory Model?

> Uptake of Dopamine normally occurs by not only the DA neurons themselves (via the DA transporter molecules) but also by glial cells. As the number of DA neurons die, the ability to uptake DA molecules also decreases, allowing the tone to maintain a constant level. However, once the level is low enough (below 80% innervation- neuron death) the glial cell uptake impacts the DA tone in the intracellular space.

> Once DA uptake (combined uptake between DA neurons & glial cells) is greater than dopamine release, DA tone decreases, and Parkinson's Patients begin to see effects of the disease.

11. As you work through the **Passive Stabilization Compensatory Model** section, fill in following table as a way to compare two theories as to why Parkinson's patients don't show symptoms until they've lost 80% of their DA neurons!

Increased Release Compensatory Model Dr. Zigmond's Theory	Agree	Passive Stabilization Compensatory Model Dr. Garis' Theory
DA tone is maintained even through neuron loss because more DA is released from each remaining neuron to make up for the neurons that have been lost.	DA tone is maintained in the striatum by the diffusion of DA molecules from regions of functioning neurons to nonfunctioning neurons. DA has less of a chance of being taken up by a DA terminal, so it diffuses longer and farther.	DA levels are maintained in a passive (not active) manner. DA levels are maintained because with the loss of DA neurons, the ability to uptake DA is also lost. Glial cells in the striatum take up DA and impact the uptake once DA neurons significantly decrease.

Passive Stabilization Program

A snapshot of the Passive Stabilization Program is shown below. This image shows 100% functioning neurons.

Describe what each colored line represents.

12. Green Line (closest to the bottom, appearing to go off the page)

> The bars green bars at the bottom represent dopamine neurons (in particular, the axon terminals, where synapses are located), and the area between the bars represents the places there are no functioning neurons.

13. White Line (wavy line that crosses all other lines)

   The wavy white line shows live dopamine concentration of the area among these neurons (notice there is DA in areas that don't have synapse.

14. Red Line (horizontal straight line)

   The red line represents the **average** concentration of dopamine in the extrasynaptic space (outside of the synapse). Also called dopamine tone.

The screen shot below shows 50% loss of DA neurons. Notice that the dopamine tone (the red straight line) is exactly the same, although the total number of DA neurons (the green bars) is halved.

Bucket Theory Analogy

15. What does each part of the Bucket Analogy represent?

a. The water faucet

Water from the faucet represents the release of DA from DA neurons

b. Large central drain

   The uptake of DA by DA neurons

c. Small drain

The uptake of DA by glial cells

d. Level of water in the bucket

   Represents the overall dopamine tone

16. How are mathematical/computer models helpful to scientists studying neurobiology?

Being able to visually represent the multiple layers of data, in real time helps biologists to study the impact that cellular influences (like death of DA neurons) have on the entire anatomical area.

17. In the Bucket Theory Animation, what changes occur to the bucket when DA neurons die?

The diameter of the water coming out of the faucet decreases, as does the large central drain. (The small drain representing the glial cells does not change.)

18. What changes occur to the bucket that account for why DA tone goes down only below 20% denervation?

The water level remains at the same height even as the faucet (DA neurons) and central drain (DA uptake) diameters become smaller. However, the once insignificant diameter of the small drain (glial uptake) becomes more impactful once the diameter of the water and large drain are reduced. The water level begins to drop as the two drains overpower the amount of water released from the faucet.
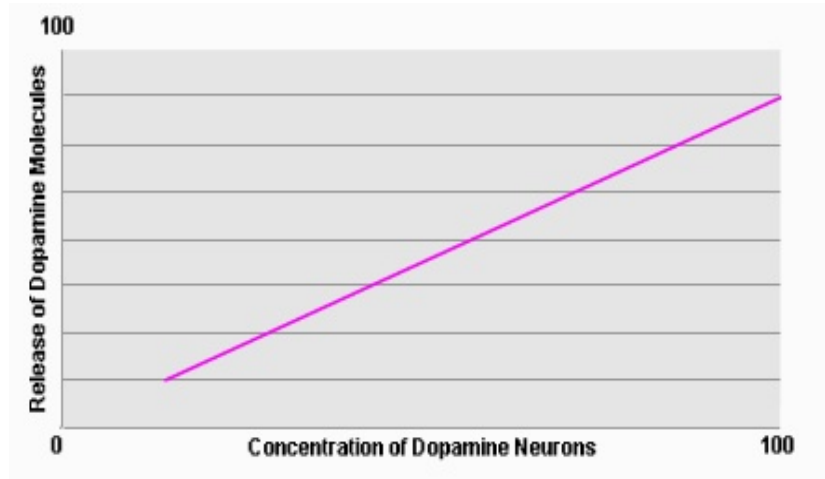
3-Way Comparison

Here are screen shots of the 3 models side-by–side at 100% innervation, 80%, 50%, 20 and 10%.
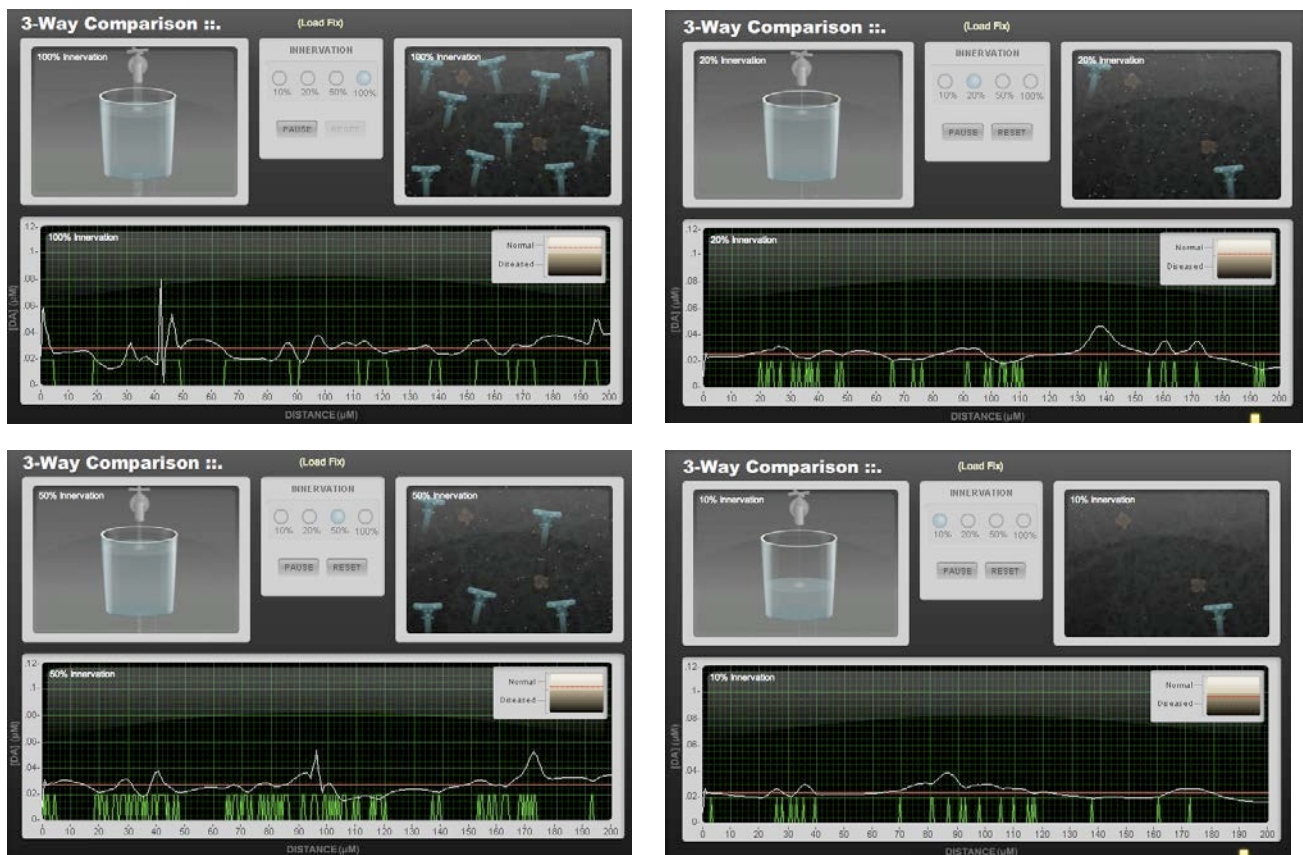
Quiz: Lab Results

1. According to the data above [to the right], what happens to the rate of dopamine release as the number of dopamine neurons decreases?

    A. DA release increases

    B. *DA release decreases

    C. DA release is unchanged

2. According to the data above (to the right) what happens to the rate of dopamine uptake as the number of dopamine neurons decreases?

    A. Uptake increases

    B. *Uptake decreases

    C. Uptake stays the same

Measure of Dopamine Uptake in the Striatum of Lesioned Rats
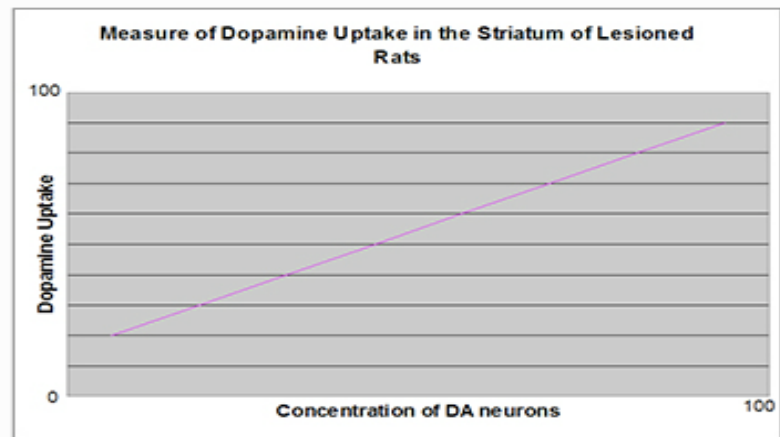
3. Based on the data above [below], which of the following conclusions can be drawn?

    A. The remaining dopamine neurons do release additional dopamine, explaining why dopamine tone is maintained despite the loss of neurons.

    B. *The remaining dopamine neurons do not release additional dopamine, which means something else must explain why dopamine tone is maintained when neurons die off.
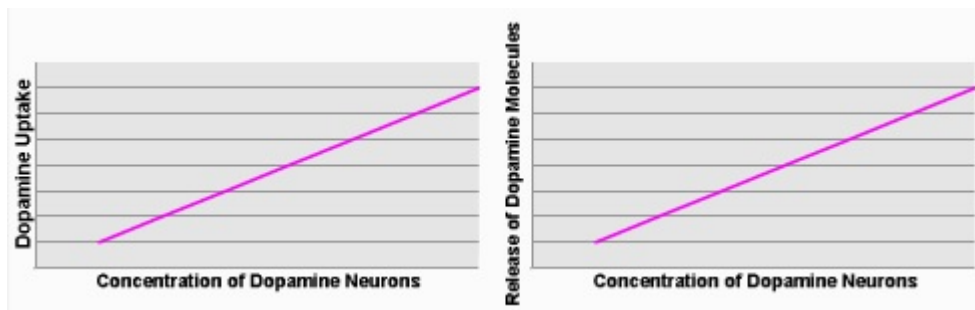
4. Based on the data above, which of the following conclusions can be drawn?

    A. Dr. Zigmond's theory is supported by the data.

    B. *Dr. Zigmond's theory is refuted by the data.

Quiz: Passive Stabilization Simulation

1. According to the passive stabilization theory, what happens to a dopamine molecule after it is released?
   A. It gets taken up by a Dopamine Transporter Molecule (DAT)
   B. It gets moved along to other parts of the brain.
   C. It gets taken up by a glial cell
   D. *A & C are both possible

2. According to the passive stabilization theory, how is dopamine tone maintained as neurons are lost?
   A. More dopamine is released with each action potential
   B. The dopamine neurons fire more often
   C. *Less dopamine is taken up by the DA neurons
   D. All of the above

3. According to the Passive Stabilization Theory, why does dopamine tone begin to drop once over 80% of the DA neurons are lost?
   A. Dopamine release is greater than dopamine uptake
   B. The amount of dopamine each DA neuron releases goes down
   C. *Dopamine uptake is greater than dopamine release because of the glial cells
   D. The amount of dopamine each DA neurons takes up increases

Quiz: Passive Stabilization Program

1. Which statement best summarizes the data generated by the passive stabilization computer model?

   A. The running DA concentration remains the same, no matter what the innervation of DA terminals
   B. The running DA concentration drops in direct proportion to the innervation of DA terminals
   C. The running DA concentration decreases after a loss of 50% of the DA terminals
   D. *The running DA concentration remains relatively constant until there are only 10% of the DA terminals remaining.

Quiz: Bucket Theory Analogy

1. The diameter of the faucet in the Bucket analogy represents...
   A. Dopamine tone in the striatum

B. *Release of dopamine by dopamine neurons

C. Uptake of dopamine by glial cells

D. Uptake of dopamine by dopamine neurons

2. The diameter of the central drain in the Bucket analogy represents…

A. Dopamine tone in the striatum

B. Release of dopamine by dopamine neurons

C. Uptake of dopamine by glial cells

D. *Uptake of dopamine by dopamine neurons

3. The drain on the right side of the Bucket analogy represents…

A. Dopamine tone in the striatum

B. Release of dopamine by dopamine neurons

C. *Uptake of dopamine by glial cells

D. Uptake of dopamine by dopamine neurons

4. As DA innervation decreases, the diameter of the faucet ____.

A. Increases

B. *Decreases

C. Stays the same

5. As DA innervation decreases, the diameter of the central drain ____.

A. Increases

B. *Decreases

C. Stays the same

6. As DA innervation decreases, the diameter of the side drain ____.

A. Increases

B. Decreases

C. *Stays the same

7. When does the amount of tone in the "bucket" decrease?

A. Any time DA innervation is lower than 100%

B. When DA innervation is at 50%

C. When DA innervation is at 20%

D. *When DA innervation is below 20%

8. What causes the amount of tone in the bucket to decrease?

A. An increase in uptake of DA by the DA neurons

B. *The impact of the glial cells taking up dopamine

C. An decrease in dopamine release by glial cells

D. None of the above

Teacher's Answer Key…

Word Drag: Read the following paragraphs, and use the word banks to fill in each blank. This matches the assessment in the virtual lab.

Parkinson's disease is a **Degenerative**, neurological disorder of unknown causes. Early symptoms include a **Resting Tremor** (trembling of the hand while it's at rest) and **Rigidity** (muscle stiffness). Parkinson's disease is considered a **Motor** disorder as it affects the movement of the body.

Patients with Parkinson's disease suffer from the loss of **Dopamine** neurons in the **Substantia Nigra**. Known as the nigrostriatal pathway, these neurons have their cell bodies in the substantia nigra. Their axons run through the **Mediastianatal Bundle** and end in the **Striatum**.

When dopaminergic neurons release dopamine, the dopamine molecules will **Diffuse** until they are taken up by a **Dopamine Transporter** or attach to a target cell. This is known as **Volume Transmission**.

Volume transmission results in a background concentration of dopamine, known as **Dopaminergic Tone**. This allows for signals to pass smoothly from the **Thalamus** to the **Motor Cortex** and then out to the rest of the body.

Parkinson's disease patients do not show visible symptoms until **80%** of the dopaminergic neurons in the striatum have died. Scientist don't know how dopaminergic tone is maintained until then. Dr. Zigmond has put forth the **Compensatory Release** model, which theorizes that the remaining dopaminergic neurons release **More** dopamine with each action potential.

Dr. Garris disagrees with this theory. He has proposed the **Passive Stabilization** model, which theorizes that dopaminergic tone is maintained because of the relationship between dopamine release and **Uptake**. As a patient loses dopamine neurons, he or she not only loses a source of dopamine **Release**, but also a source of dopamine uptake. Therefore, the net concentration of dopamine molecules remains the **Same**. This stabilization is disrupted as the **Glial** begin to compete with the dopaminergic neurons for the uptake of dopamine molecules.

Original Resources on Which the Lab is Based

The Virtual Parkinson's Lab is based on real research, done by real scientists. Hopefully, by participating in this lab, students can see how knowledge is furthered by the publication of research. One scientist shares what he/she has learned from conducting experiments and speculates about the implications that this data has for our broader knowledge. Other scientists can repeat those some experiments or modify them to determine whether their data support the same conclusions. In this case, we have two opposing explanations of how dopamine tone is maintained in preclinical PKD patients. Here are the journal articles on which this lab is based. The abstracts were written by the author's themselves as part of the publication. They are very technical but students who have completed our lab will understand a surprising percentage of the complete abstract. Dr. Paul Garris was the technical advisor on this virtual lab.

Dr. Zigmond's research

Zigmond M. J., Abercrombie E. D., Berger T. W., Grace A. A. and Stricker E. M. (1990) Compensations after lesions of central dopamine neurons: some clinical and basic implications. *Trends Neurosci.* **13**, 290–296.

Abstract

Parkinson's disease is associated with degeneration of the dopamine component of the nigrostriatal pathway. However, the neurological symptoms of this disorder do not emerge until the degenerative process is almost complete. A comparable phenomenon can be observed in animal models of Parkinson's disease produced by the administration of the selective neurotoxin, 6-hydroxydopamine (6-OHDA). Studies using such models suggest that the extensive loss of dopamine neurons is compensated, in large part, by increased synthesis and release of dopamine (DA) from those DA neurons that remain, together with a reduced rate of DA inactivation. These findings may have important implications for the diagnosis and treatment of a variety of neurological and psychiatric diseases, as well as for our understanding of plasticity in monoaminergic systems.

Dr. Paul Garris' Research

Bergstrom, B. P. and Garris, P. A. (2003), 'Passive stabilization' of striatal extracellular dopamine across the lesion spectrum encompassing the presymptomatic phase of Parkinson's disease: a voltammetric study in the 6-OHDA-lesioned rat. Journal of Neurochemistry, 87: 1224–1236. doi: 10.1046/j.1471-4159.2003.02104.x

Abstract

Symptoms of Parkinson's disease do not present until the degeneration of nigrostriatal dopamine neurons is nearly complete. Maintenance of dopamine tone governing striatal efferents is postulated to preserve motor control during the presymptomatic phase, but the neuroadaptation responsible for normalization is not completely understood. In particular, the prevailing view that surviving dopamine neurons compensate by up-regulating release has been difficult to demonstrate directly. Here we investigate dopamine neurotransmission in the hemiparkinsonian rat using fast-scan cyclic voltammetry at carbon-fiber microelectrodes. Electrical stimulation was used to elicit extracellular dopamine levels mimicking the steady-state dynamics of tonic dopamine signaling. In agreement with microdialysis studies, evoked steady-state dopamine levels remained constant over the entire lesion spectrum (0 to ~85%) observed during the presymptomatic stage. Kinetic analysis of the voltammetric recordings demonstrated that evoked dopamine concentrations were normalized without plasticity of dopamine release and uptake, suggesting that the primary mechanisms controlling ambient levels of extracellular dopamine were not actively altered. In the present study, we formalize this neuroadaptation as 'passive stabilization'. We further propose that passive stabilization is mediated by the simple physical principles of diffusion and steady state, is predicated on extrasynaptic transmission, and forms the basis for a new compensation model of preclinical parkinsonism.