The Neurobiology of Sleep

Life Sciences Learning Center

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Core Concept

Students will apply knowledge of sugar metabolism, storing of memories, cellular waste disposal, and growth hormones to understanding how sleep can affect many different body systems.

Class Time Required

Approximately 200 minutes – 3 full class periods (includes 4 hands-on laboratory experiments) and another half period. Labs should be set up to be done in rotating group

Supplies You'll Need

for 30 students working in groups of 2, each experiment set up as four stations (Can be used for up to 2 consecutive classes:

76 3oz plastic cups
20 plastic droppers
30 2x3inch pieces of chromatog raphy (or cardstock) paper
4 black lights (optional)
8 small white plastic plates (like weigh boats)
52 Cotton swabs

150mL pH 12 Buffer solution
30mL pH 9 Buffer solution
70mL 0.5% Phenolphthalein Solution
100mL Tonic water with quinine
A pinch of table salt (NaCl)
20g Baking soda
25mL 0.1% Bromothymol Blue
200mL White Vinegar

(Buffers, phenolphthalein, Sodium Hydroxide and bromothymol blue solutions can all be bought from Wards Natural Science)

Solutions You'll Need to Make

Tonic water and salt:
80 mL Tonic Water
A pinch of salt (NaCl), you're just trying to add grains of salt until the fluorescence is about half as dim as the regular tonic water.

70% White Vinegar
30mL water
70mL White Vinegar

"NBT & ROS Solution"
225mL water
20g Baking Soda
25mL 0.1% Bromothymol Blue

Ordering Information (From Wards)

Chromatography paper
#15W3708 50 sheets (6" X ¾") $5.10

pH 12.00 Buffer Solution
#9405206 500mL bottle $13.15

pH 9.00 Buffer Solution
#9406206 500mL bottle $13.15

0.5% Phenolphthalein Solution
#9511406 500mL bottle $6.75

0.1% Bromothymol Blue
#9447106 500mL bottle $11.30

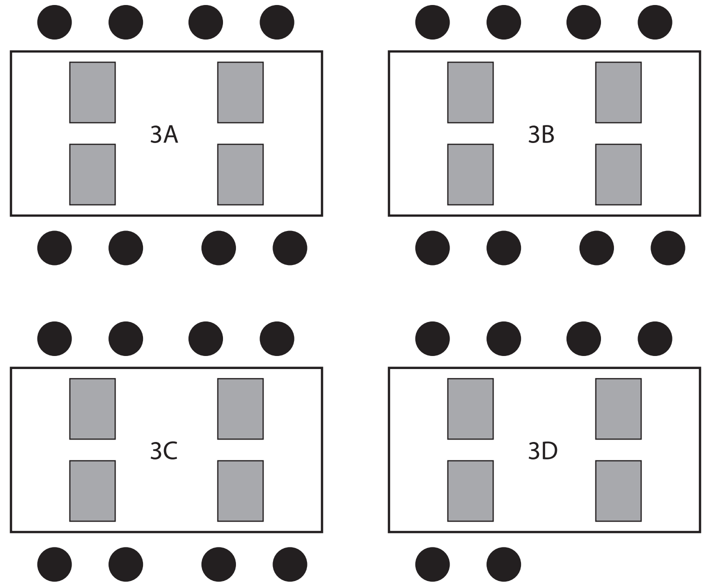
0.1M (0.4%) Sodium Hydroxide
#9707806 500mL bottle $5.25,

0.5% Phenolphthalein Solution
#9511406 500mL bottle $6.75

Laboratory Set-up

There are four experiments in this lesson (3A, 3B, 3C and 3D). Rather than set up all four experiments for each group of two students, we recommend setting each experiment up as a station with four “placemats” (all the supplies needed to do one experiment) at each station. Shown to the right is an example of a laboratory that is set up for 30 students working at four stations. Students can rotate throughout the stations. Some stations take longer than others, but students can be writing in their lab notebooks, or preparing for the next station while waiting.

The instructions for setting up a single placemat at each station are shown below. Placemats can be printed out and slid into sheet protectors



Experiment 3A Placemat Setup

7 3oz plastic cups labeled and filled as follows:

- “N10” = 5mL pH 12 buffer
- “N40” = 5mL pH 9 buffer
- “N100” = 5mL water
- “R10” = 5mL pH 12 buffer
- “R40” = 5mL pH 12 buffer
- “R100” = 5mL water
- “Glucose Detection Fluid” =5mL 0.5% Phenolphthalein solution

Place a plastic dropper into the detection fluid
Place cotton swabs in all the other cups
15 2x3 chromatography papers

Experiment 3B Placemat Setup

4 15mL tubes, labeled and filled as follows:

- “Control 1” = 5mL Tonic water
- “Control 2” = 5mL Tonic water and salt
- “Group 1” = 5mL Tonic water and salt
- “Group 2” = 5mL Tonic water

1 black light

**If no black light is available, print out the “Synapse Counting Sheets” on page 15

If using a black light, make sure this is set up in a darker or shadowed area of the room.

Experiment 3C Placemat Setup

3 3oz plastic cups labeled and filled as follows:

- “Control Brain sample” = 10mL White vinegar
- “Experimental Brain sample” = 10mL 70% white vinegar
- “NBT & ROS solution” = 10mL NBT & ROS Solution

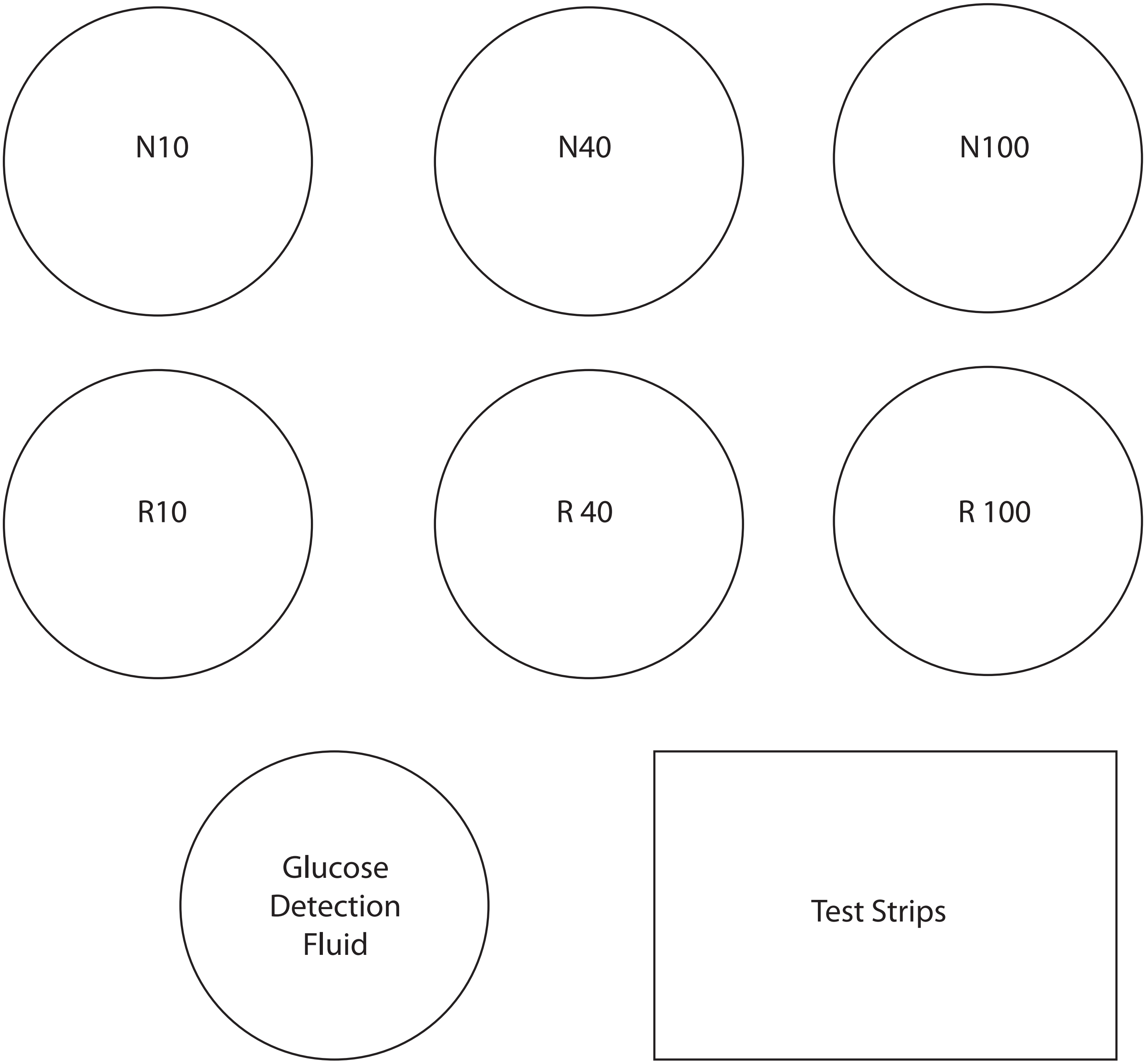
Place a plastic dropper into each cup
2 small plastic dishes

Experiment 3D Placemat Setup

8 3oz plastic cups labeled and filled as follows:

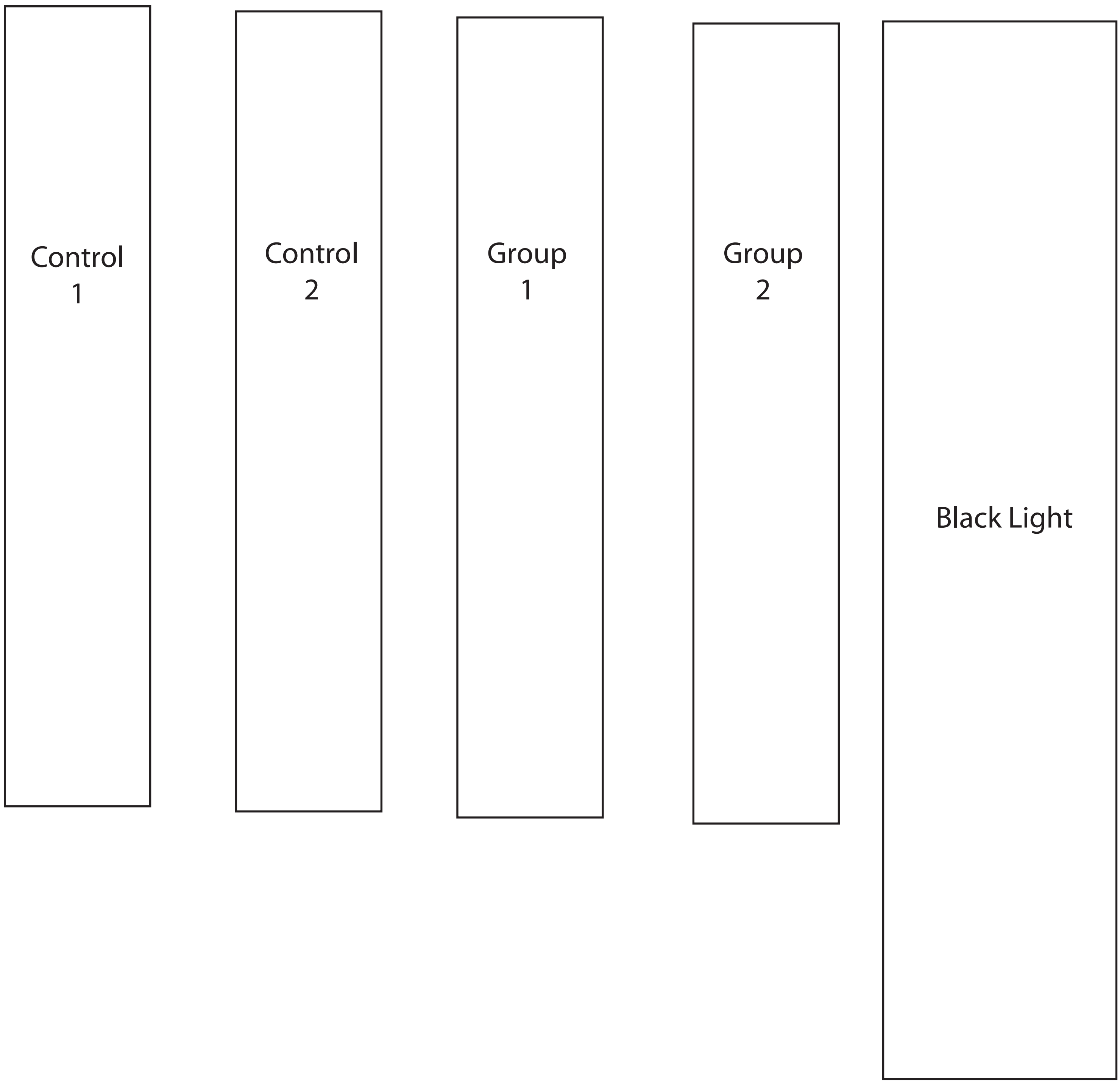
- “7pm” = 5mL water
- “9pm” = 5mL water
- “11pm” = 5mL water
- “1am” = 5mL water
- “3am” = 5mL water
- “5am” = 5mL water
- “Positive Control” = 5mL pH 12 buffer
- “Detection Fluid” = 5mL 0.5% Phenolphthalein solution

Place a plastic dropper into the detection fluid
Place cotton swabs in all the other cups
15 2x3 chromatography papers



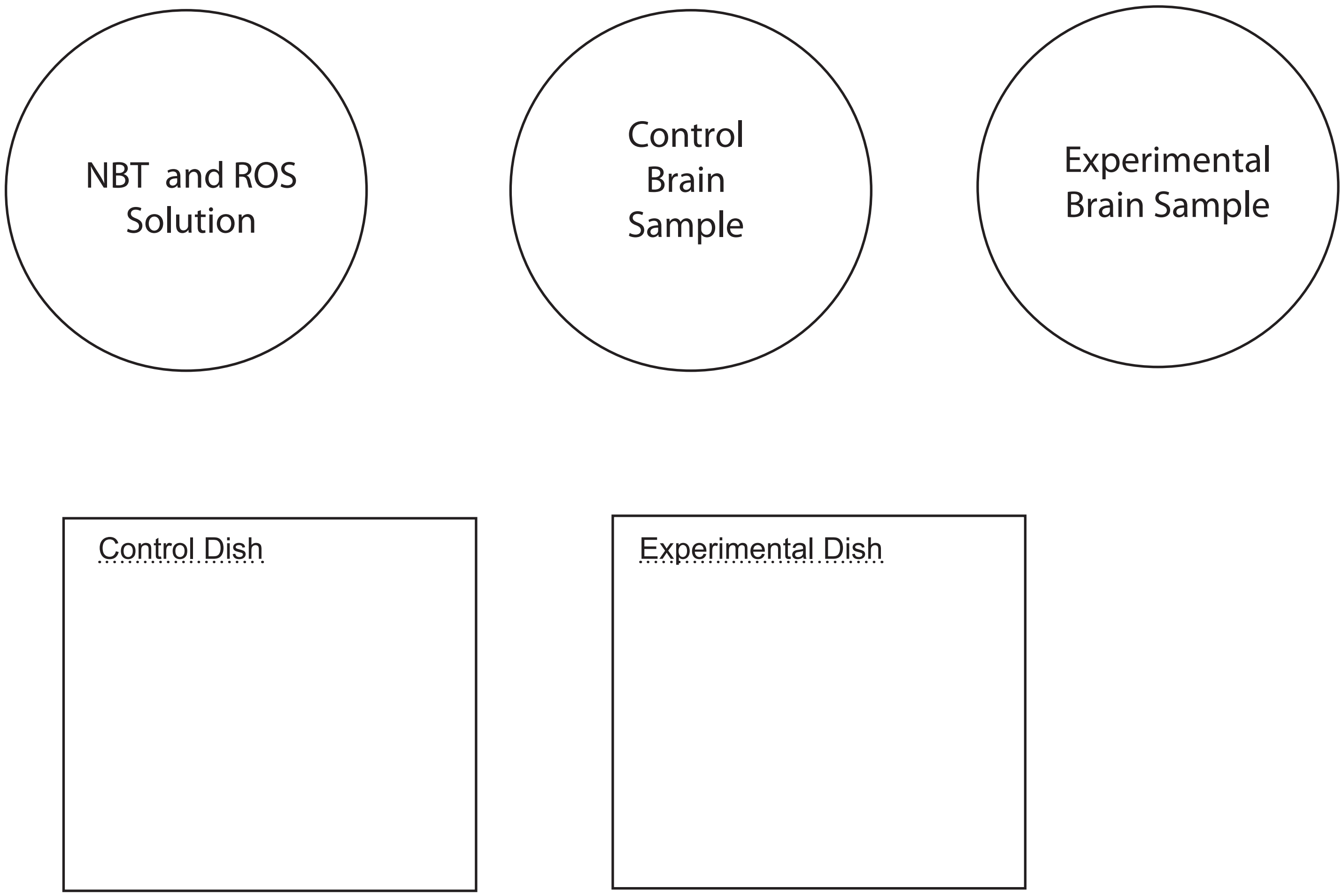
3A Glucose Tolerance Test Placemat

Follow all the directions in your hand-out for how to perform this experiment. Keep all droppers and swabs in their proper containers, do NOT mix. Discard your test strips when finished.



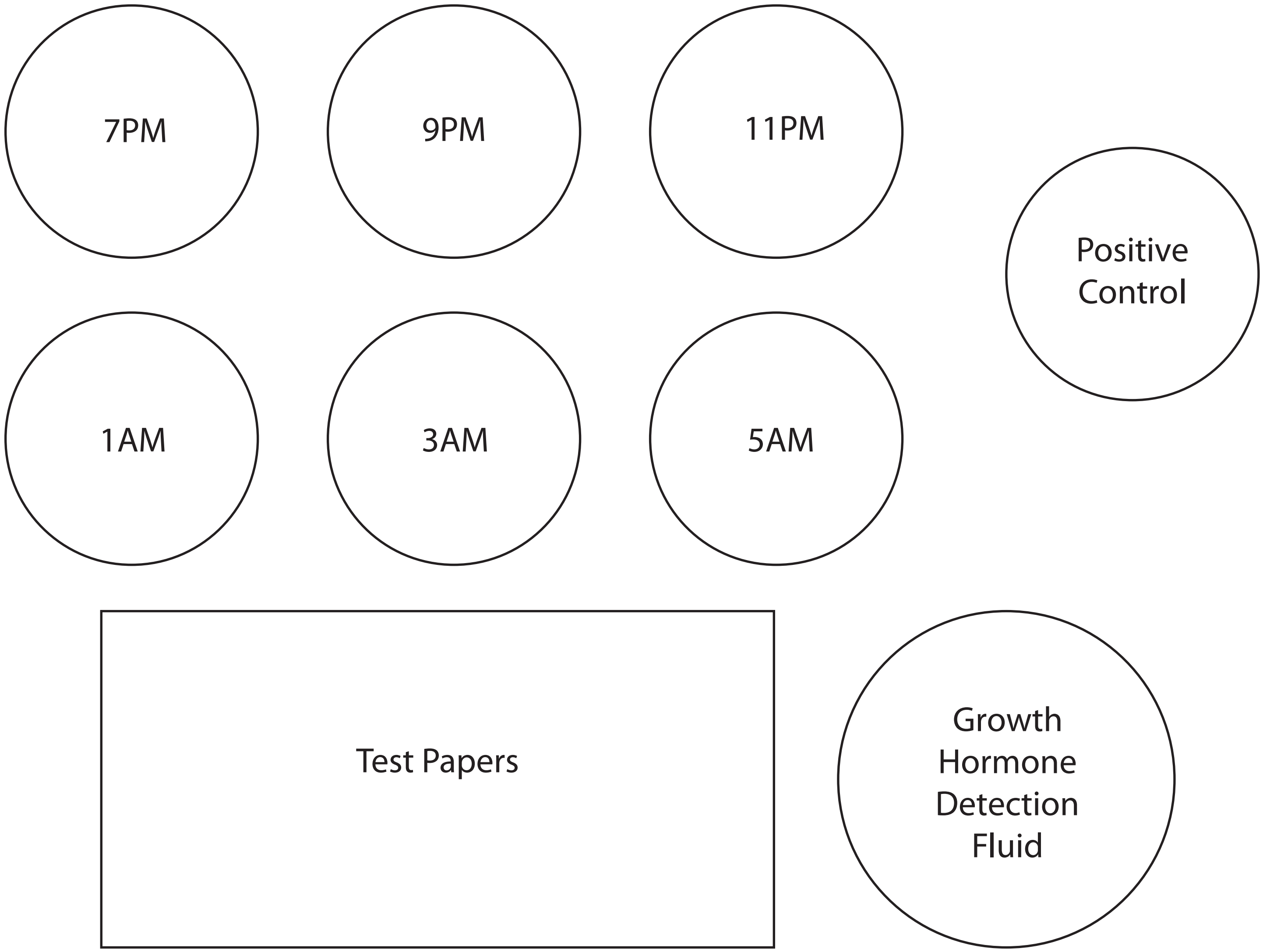
3B Synapse Counting Placemat

Follow all the directions in your hand-out for how to perform this experiment.



3C Sleep and Cellular Wastes Placemat

Follow all the directions in your hand-out for how to perform this experiment. Keep all droppers in their proper containers, do NOT mix droppers. RINSE OUT YOUR DISH WHEN YOU ARE FINISHED!



3D Growth Hormone and Sleep Placemat

Follow all the directions in your hand-out for how to perform this experiment. Keep all droppers and swabs in their proper containers, do NOT mix droppers or swabs. Discard your test strip when finished.

Suggested Class Procedure

Optional activity to be started a week before starting this lesson:
Have students keep a sleep diary for a week to get a better idea of when they are sleeping and how well they are sleeping. The National Sleep Foundation has a sleep diary worksheet that can be found at http://www.sleep.buffalo.edu/sleepdiary.pdf

Because the student hand-out has so many pages, we recommend printing out class sets of the hand-outs that can be reused, and having students keep their own lab notebooks in which they answer questions and draw their results. Questions are numbered based on what part they are in the booklet.

Class period 1
1. Distribute a copy of the student booklet. Ask students not to read ahead.
2. Have students read parts 1 and 2 and answer the questions in their lab notebooks.

Class period 2
3. Go through answers to questions and provide interpretation for questions 2.1-2.5

- Answering "yes" to questions 3 or 4 indicates that you are not a short sleeper
- In comparing the answers to questions 1 and 2, if you sleep more on weekends or vacations, you are not a short sleeper
- If you are not a short sleeper and you sleep less than 8 hours a night on a regular basis, you are considered "sleep deprived."

4. Read through Part 3 together with students. Have them answer questions 3.1-3.6 in their lab notebooks.
5. Prepare a lesson summary chart (See example on page 14) that will be used to summarize what students learn and find out from their labs.
6. Read (out loud, or individually) the introductions of each activity and answer the questions that follow each introduction. Have students develop a one sentence summary of each introduction to place in the lesson summary chart.
7. Have students read The Study section and develop a one sentence summary to place in the lesson summary chart.
8. Have students develop their hypothesis in their lab notebooks. On the summary chart, summarize their hypothesis by having a "not sleeping will change this, agree or disagree" section where students can indicate whether they agree or disagree.

Class period 3:

9. Have students read (or read together) the instructions for doing each lab. Allow students to work in groups of 2, and rotate them through each station (about 10 minutes per station) and fill in their data and answer the questions in their lab notebooks.

Class period 4

10. Have students give a one sentence summary of their conclusions to be put on the summary chart.
11. Have students read Part 4 (A Mayo Clinic article on teens and sleep).
12. Together, or individually, have students answer the questions in part 4.

Sample Student Lab Notebook (Answer Key)

Part 1

1.1) I think the real life-benefits of not needing any sleep are that I would have more time to get things done and do fun things.

1.2) I think the disadvantage of not needing any sleep is that I might get bored if other people are sleeping

Part 2

2.1) I sleep about 7 hours a night on weeknights

2.2) I sleep about 11 hours a night on weekends

2.3) I wish I could sleep more. Sometimes I almost fall asleep in class.

2.4) I take naps when I get home.

2.5) I'm not a short sleeper because I take naps, I wish I could sleep more, and I sleep more on weekends when I have a chance to sleep in.

Part 3

3.1) Yes, I want to sleep less!

3.2) I think it's enough, except that I'm still tired sometimes.

3.3) No.

3.4) 8 hours on weeknights and 12 hours on weekends.

Part 3A

3.A.1) Liver, fat and muscle.

3.A.2) Higher.

Part 3B

3.B.1) Increase.

3.B.2) It removes some synapses.

Part 3C

3.C.1) ROS, reactive oxygen species. Superoxides. Hydrogen Peroxide.

3.C.2) Antioxidants or superoxide dismutase (SOD)

Part 3D

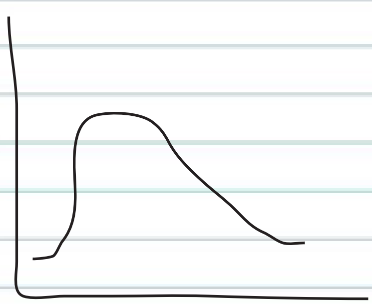
3.D.1) Age, gender, diet, exercise, stress or other hormones.

3.D.2) It makes you taller, increases muscle mass, strengthens bone and stimulates the growth of organs (except for the brain).

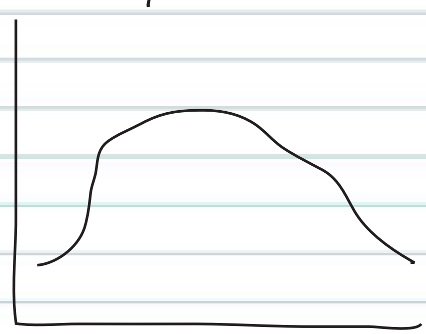
Hypotheses:

3.A.Hypothesis): Sleep restricted is insulin resistant.

Normal



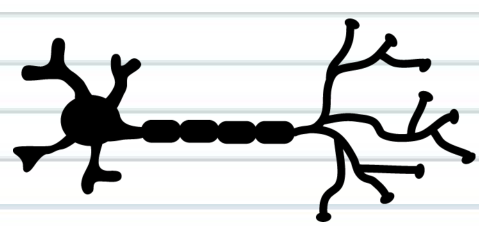
Sleep Restricted



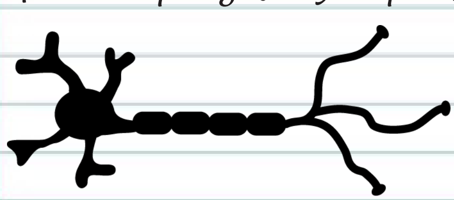
3.B.Hypothesis

After learning (9synpases)

After not sleeping (9 synapses)



After sleeping (3 synapses)



They will look the same, because if you don't sleep, you'll still be not learning, so you won't get any more, and you'll probably get less.

3.C.Hypothesis: A mouse that is sleep deprived probably produces the same amount of SOD compared to a mouse that sleeps normal, because they have the same amount of cellular wastes.

3.D.Hypothesis)

	Rats sleeping	Sleep deprived rat
7:00pm	0ng/mL GH	0ng/mL GH
9:00pm	500ng/mL GH	0ng/mL GH
11:00pm	0ng/mL GH	0ng/mL GH
1:00am	0ng/mL GH	0ng/mL GH
3:00am	0ng/mL GH	0ng/mL GH
5:00am	0ng/mL GH	0ng/mL GH

DATA

3.A.Data table

	Normal Sleep (10 hours)	Sleep Restricted (5 hours)
10min	Dark pink	Dark pink
40min	Lighter pink	Dark pink
100 min	No color	No color

3.A.3) Decreased.

3.A.4) He might end up being insulin resistant which would mean he might get fatter than if he was getting enough sleep.

3.B.Data table

Control 1	Control 2	Group 1	Group 2
Bright	Dull	Dull	Bright

3.B.3) Yes. The flies that spent 12 hours in the mall have more active synaptic terminals than the ones that were in the vial.

3.B.4) Yes. The flies that were in the mall, then slept have fewer terminals after sleep.

3.B.5) No. They had the same amount of active synaptic terminals.

3.B.6) Yes, sleep is important because you only have less terminals after you sleep.

3.B.7) A fly that doesn't sleep won't remember things as well.

3.C.Data table)

	CONTROL	EXPERIMENTAL
SOD activity	About 30 drops	About 40 drops

3.C.3) There was less SOD in the sleep deprived mice...I had to add more to make the solution turn yellow.

3.C.4) There would be more waste in the mouse's brain. They might start to get brain damaged.

3.D.Data Table)

	Rats sleeping	Sleep deprived rat
7:00pm	0ng/mL GH	0ng/mL GH
9:00pm	500ng/mL GH	0ng/mL GH
11:00pm	0ng/mL GH	0ng/mL GH
1:00am	0ng/mL GH	0ng/mL GH
3:00am	0ng/mL GH	0ng/mL GH
5:00am	0ng/mL GH	0ng/mL GH

Yes, the positive control worked

3.D.3) The sleep deprived rat produce no growth hormone compared to rats that sleep normally

3.D.4) Sleep deprived rats would probably be pretty small and weak compared to rats that slept normally.

Part 4

4.1) Not getting enough sleep can make it harder for you to metabolize sugar. It stays in your blood for longer and makes you insulin resistant. It can make you fat.

4.2) Not getting enough sleep might make you not remember things as well because your neurons can't organize.

4.3) Not getting enough sleep can make toxic wastes build up in your brain and cause brain damage.

4.4) Not getting enough sleep can make you smaller because your body doesn't produce enough growth hormone.

4.5) Maybe short sleepers can do all the things that people who sleep normally can do, they just do it all faster. Or maybe their bodies are just healthier so that even if all these problems happen, it doesn't really make them small or remember less.

4.6) 9 hours a night

4.7) I think 8 is fine

4.8) No, I like to sleep

4.9) I still only get 7hours

4.10) I still like to sleep in…11 hours

4.11) It's probably not enough sleep.

4.12) There are health effects to not getting enough sleep

4.13) I used to think it was enough sleep, but I think I'm probably doing bad things to my body by not sleeping enough.

4.14) I could go to bed earlier.

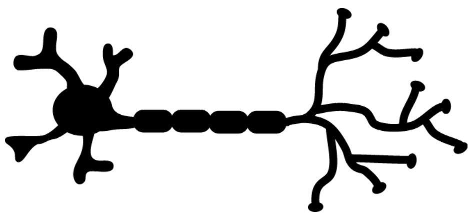
Sample Class Summary Chart

	Metabolism of sugar 3A	Memory (synapses) 3B	Cellular Waste Removal 3C	Growth Hormones 3D
Summarize Intro	Insulin tells us to store sugars in our muscle and liver. If we are insulin resistant, we tend to store it in fat	When we learn things, we make lots of synapses. Then we trim down the number of synapses to keep our brains organized	We have to get rid of cellular wastes like ROS, otherwise our cells die. SOD is an enzyme that does this.	Growth hormones make us big and strong.
What did the study compare?	Comparing men who slept 5 vs 10 hours	Comparing flies that went to a mall and slept versus didn't sleep	Measuring the amount of SOD in mice that slept vs didn't sleep	Measuring the amount of GH in mice that slept versus didn't sleep
Vote! Does sleep matter?	Does sleep matter? 10 agree 20 disagree	15 agree 15 disagree	5 agree 5 disagree	30 agree 0 disagree
What did the study show?	Men who didn't sleep are insulin resistant, men who slept are normal.	Flies that didn't sleep didn't organize their synapses like the flies that slept	Mice that didn't sleep have less SOD...probably more cellular waste	Mice that didn't sleep didn't make any growth hormone.

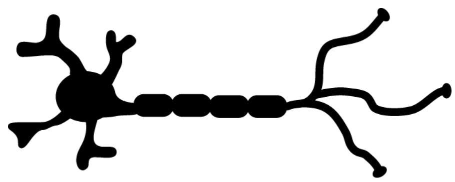
DATA SHEETS FOR EXPERIMENT 3B (SYNAPSE COUNTING) IF NO BLACK LIGHTS ARE AVAILABLE

Researchers have taken computer images of the neurons taken from each of the flies in the two experimental groups and the control group. Examine these images:

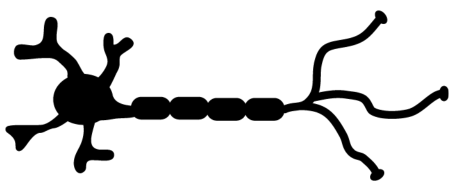
Control 1: Fly mall



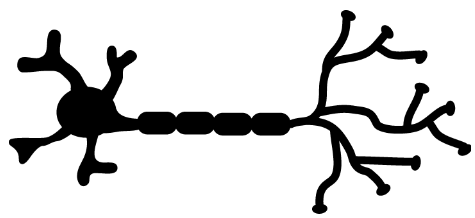
Control 2: Vial



Group 1: Fly mall, then sleep

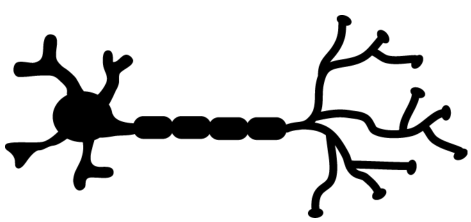


Group 2: Fly mall, then awake

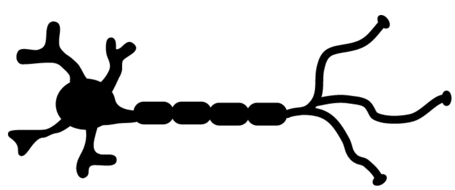


Researchers have taken computer images of the neurons taken from each of the flies in the two experimental groups and the control group. Examine these images:

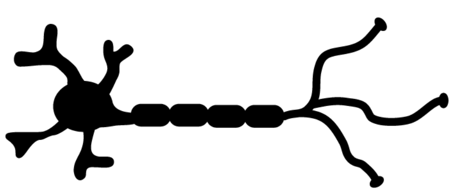
Control 1: Fly mall



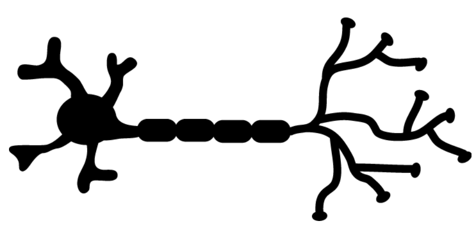
Control 2: Vial



Group 1: Fly mall, then sleep



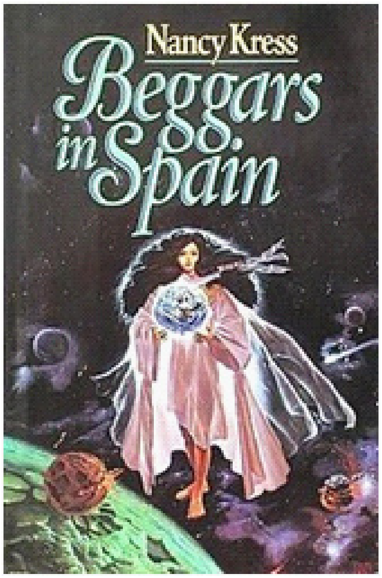
Group 2: Fly mall, then awake



Sleep

A necessity of life, or a waste of time?

PART 1: Sleep in Fiction, the Rise of the Sleepless Genemods?



Beggars in Spain (and its sequels), written by Nancy Kress, takes place in a future where genetic engineering has become a reality, and children born with genetic changes are called “genemods.”

Leisha Camden is the twenty-first human being to have the gene for sleeplessness. She is the daughter of financier Roger Camden, who felt he had wasted far too much of his life in sleep, and his wife Elizabeth Camden, an Englishwoman who wanted a normal child. Sleeplessness confers a number of secondary benefits—higher IQ and a sunnier disposition, as well as 1/3 more productive time (vs the time the unmodified spend asleep).

Sleepless not only don’t need sleep, they cannot sleep. Of the original twenty-one, twenty grow up to be well-adjusted, intelligent, capable children. The nineteenth child illustrated some of the drawbacks when it was accidentally shaken to death by sleep deprived “normal” parents who could not cope with a baby who was awake and active 24 hours a day.

By the age of fifteen Leisha has become a part of the community of Sleepless, few though there are in the world; she, like all of them, is several grades ahead of her age. The oldest, Kevin Baker, has already become a wealthy computer software designer. Tony Indivino’s mother had problems adjusting to his Sleepless ways and forced him to live as a “Sleeper.” He predicts that the Sleepers will soon begin to discriminate against Sleepless, and is quickly proved right: a Sleepless athlete is barred from the Olympics, for instance, because her 16-hour practice days are impossible for other competitors. Some cities forbid Sleepless from running “24-hour” convenience stores. “

Adapted from http://en.wikipedia.org/wiki/Beggars_in_Spain

“I’m so tired, I could DIE...”

In real life, there are an extremely small number of people who suffer from a genetic disorder of the protein PrP^{c} who actually can say that and mean it. Found in just 40 families worldwide, the PrP^{c} mutation causes a dominant disorder called Familial Fatal Insomnia.

People with this mutation typically don’t show any symptoms until later in life, often after having children of their own. Once the symptoms start (an inability to sleep, leading to panic attacks and paranoia) the individual typically has only 7-18 months to live. During that time, he or she never sleeps.

QUESTIONS

1.1) What do you think are the real-life benefits of not needing any sleep?

1.2) What do you think would be the disadvantages of not needing any sleep?

PART 2: Is Sleeplessness Just Science Fiction?

Maybe not…in 2011, the Wall Street Journal published an article describing the “Sleepless Elite.” These people represent 1-3% of the population who are both “night owls and early birds.” As described in the article:

> They typically turn in well after midnight, then get up just a few hours later and barrel through the day without needing to take naps or load up on caffeine. They are also energetic, outgoing, optimistic and ambitious, according to the few researchers who have studied them.
>
> The pattern sometimes starts in childhood and often runs in families. Researchers have even identified a single gene mutation (called hDEC2) that was shared by a mother and daughter who were short sleepers. When mice have this same mutation, they sleep less than normal mice. But researchers don’t think there are just one or two genes that regulate sleep. It’s likely that there will be many different genes that all play a part in the complex, and not-well-understood system that regulates sleep.

True short sleepers are rare and seem to share certain characteristics. They typically have a more cheerful personality, their metabolism is higher, and they have a high tolerance for physical pain and psychological setbacks. As one researcher said “there is some sort of psychological and physiological energy to them that we don’t understand.”

Thomas Edison, the sleepless elite?

Thomas Edison, inventor of the light bulb, scoffed at sleep. He said, “Most people overeat 100 percent, and oversleep 100 percent, because they like it. That extra 100 percent makes them unhealthy and inefficient.” He himself was said to have slept less than five hours a day. If he was particularly busy in his lab, he wouldn’t sleep at all!

Of course, he might have been exaggerating. Those who knew him say he often took long naps, and sometimes slept all day long. Must have been a slow day in the lab…

QUESTIONS

2.1) How many hours a night do you sleep on weeknights?

2.2) How many hours do you sleep on weekends or vacations?

2.3) Do you wish you could sleep more sometimes?

2.4) Do you take naps?

Your teacher will provide you with a key to figure out if you are a short sleeper or not after you have answered these questions.

2.5) Are you a short sleeper? Why not?

PART 3: What does sleep do?

Can you train yourself to be a short sleeper? If you only let yourself get 4-5 hours a sleep a night, do you think your body might just get used to it? Before you buy a really loud alarm clock, maybe we should see what happens to people who get less sleep (4-5 hours a night).

QUESTIONS

3.1) Are you interested in training yourself to sleep less?

3.2) Do you think you get “Enough sleep?”

3.5) Do you think there are any health effects to not getting “Enough sleep?”

3.6) How much sleep do you think is “Enough sleep” for you?

You will now complete a series of laboratory studies to determine if sleep has any effects on an organism’s:

- metabolism
- memory
- ability to remove toxic cellular waste
- concentration of growth hormone

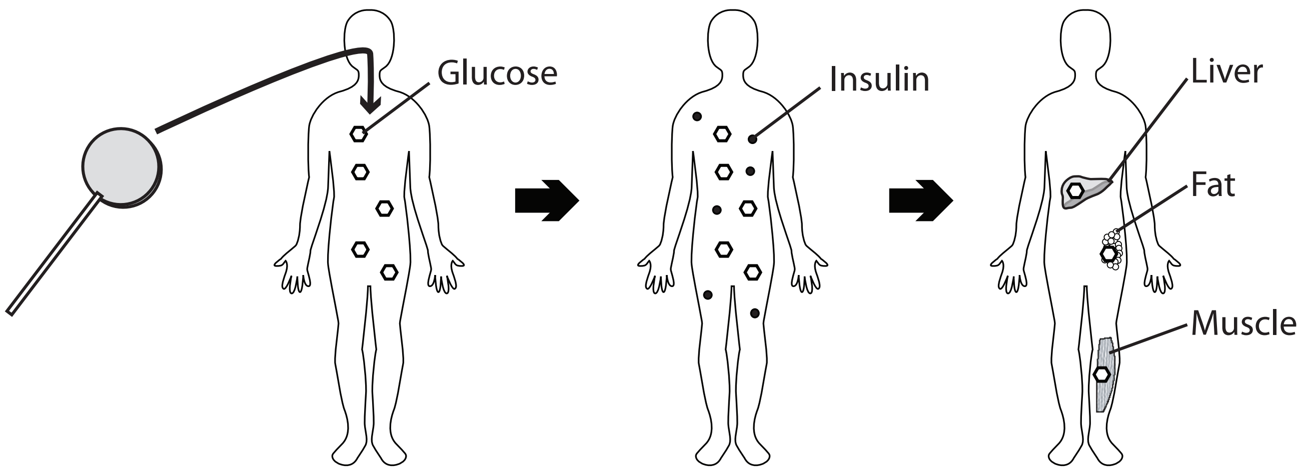
For each section:

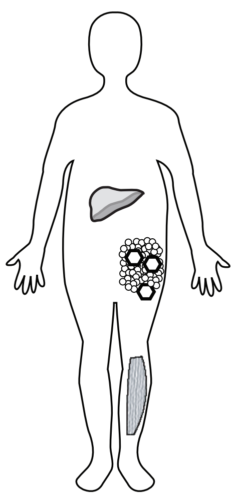
- Read the introduction.
- Read about the study. This is an experiment that was done in real life, and that you’ll be doing some lab tests on.
- Write down your hypothesis -- what do you think will happen?
- Perform the experiment.
- Record your results.
- Answer a few questions to make sure you really got it

PART 3A: How might not getting enough sleep affect your metabolism of sugar?

INTRODUCTION

Blood glucose (a type of sugar) goes up when you eat, especially when you eat things with a lot of sugar or carbohydrates, like candy or bread. Insulin is a hormone that is secreted by the pancreas when blood glucose levels are high. Insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle, and fat in fat tissue.





Some people have a problem called insulin resistance. This happens when liver and muscle cells are not as sensitive to insulin and don't take up glucose. However, fat cells tend to NOT develop resistance. This means that fat cells take up a lot of the extra sugars and convert them into fat. For this reason, insulin resistance can lead to weight gain and eventually, obesity. Insulin resistance is detected when a person's blood sugar stays higher than normal after eating.

In a nutshell: The body's ability to secrete and respond to insulin regulates body fat. Reduced sensitivity to insulin can lead to weight gain.

QUESTIONS

3.A.1) Name three organs that take up glucose when insulin is present in the body.

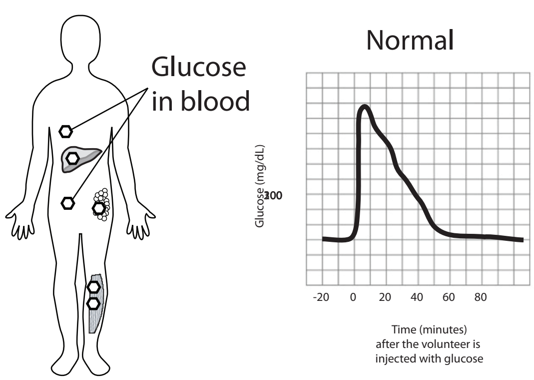
3.A.2) When a person is insulin resistant, will they have higher than normal blood sugar after eating or lower than normal blood sugar after eating?

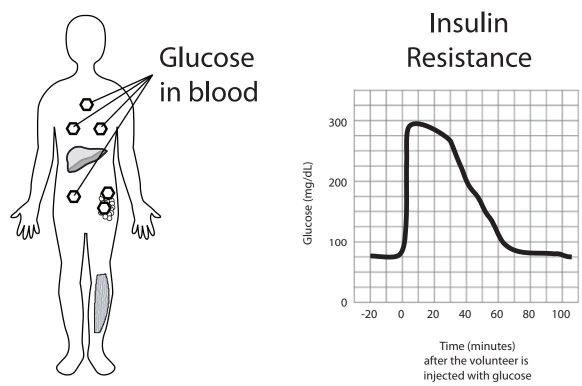
THE STUDY:

Twenty healthy people slept for 10 hours per night for eight nights (normal sleep), followed by 5 hours per night for seven nights (sleep restricted). On the last day of each condition, each person was given a glucose tolerance test.

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
		Sleep for 10 hours a night!				
8am Glucose Tolerance Test		Sleep for 5 hours a night!				
8am Glucose Tolerance Test						

In the glucose tolerance test, the volunteer is injected with a dose of glucose. Every 20 minutes after that, researchers measure the amount of glucose circulating in the volunteer's blood by taking a blood sample. A normal individual would respond to this dose of glucose by increasing his or her production of insulin. Someone who is resistant to insulin would also produce this insulin, but the insulin would not remove glucose from the blood as quickly. Therefore, someone who is insulin resistant would have higher glucose levels in his or her blood for a longer time. The results of a glucose tolerance test for a normal person and an insulin resistant person would look like this:



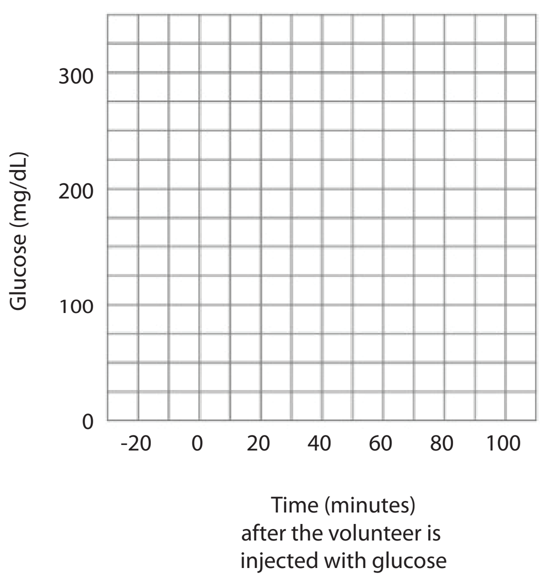


In a nutshell: A person's ability to absorb glucose can tell you if a person is resistant to insulin.

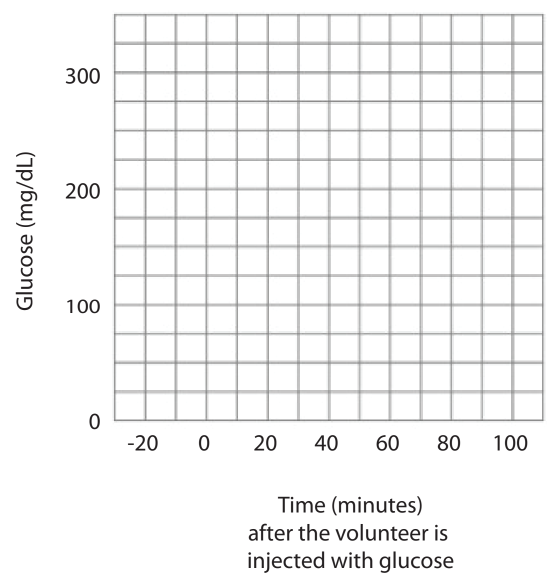
QUESTIONS

3A.HYPOTHESIS
What do you think the results of a glucose tolerance test would be for a volunteer who had slept for 10 hours a night (sleep normal) compared to someone who slept for 5 hours a night (sleep restricted)? Draw two graphs like the ones below that shows your hypothesis. Are they the same? Different?

Sleep Normal



Sleep Restricted

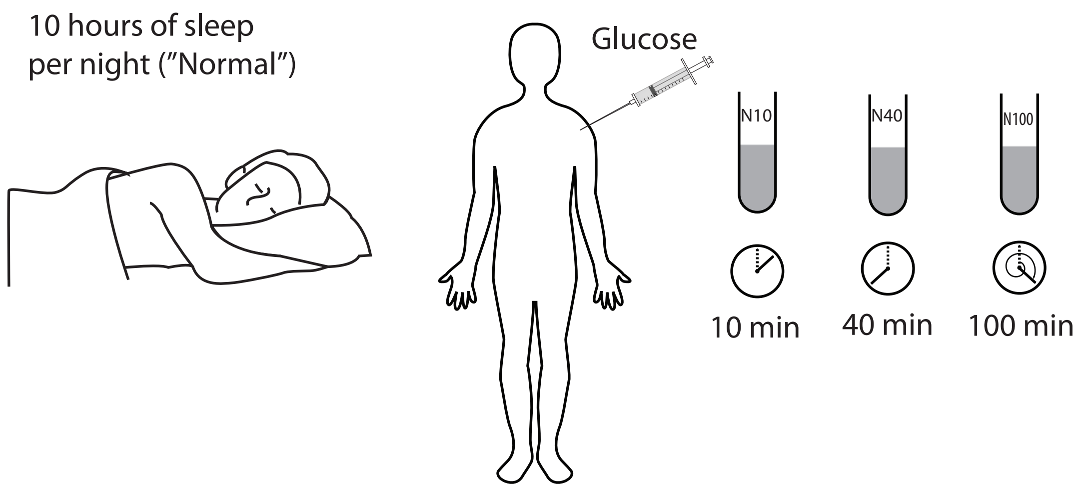


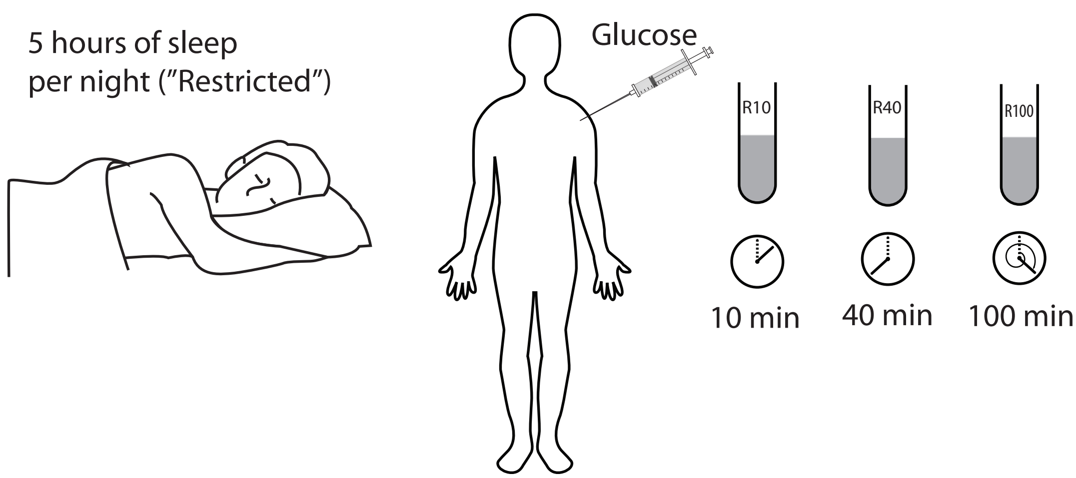
THE EXPERIMENT: Glucose Tolerance Test

You are going to test for the amount of glucose that is in the blood serum (The liquid part of blood that doesn't include red blood cells) of a volunteer 10, 40 and 100 minutes after a glucose injection.

You will be comparing a set of blood samples from when the volunteer was sleeping for 10 hours, to one where he was sleeping for 5 hours.

We can't get a quantitative result (actual numbers like 100ng/dL of glucose) using the materials we have here, but we can get an idea of whether or not a sample has MORE or LESS glucose based on relative color changes.





MATERIALS

- Normal sleep (N) set of blood samples
  - N10
  - N40
  - N100
- Sleep restricted (R) set of blood samples
  - R10
  - R40
  - R100
- Glucose detection solution
- Test paper

PROTOCOL:

1.Label the test paper as shown below using a pencil. Each circle should be about 1cm in diameter

○ N10	○ N40	○ N100
○ R10	○ R40	○ R100

2.Use a cotton swap to apply each sample in the appropriate spot (R10 onto the R10 spot, R40 onto the R40 spot, etc)

3.Allow the samples to dry for about 30 seconds.

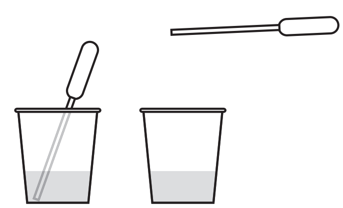
4.Apply one drop of glucose detection solution to each spot. Draw a table, as shown below. Record how dark each spot looked on the table using the color guide below

○ No glucose ○ A little glucose ● A lot of glucose

Pay attention, only use one dropper at a time!

In a forensics lab, you would never use the same pipette with more than one liquid. It might mean the difference between being found guilty or innocent!

In an effort to conserve, you may be re-using pipettors. As long as one pipettor is always being used for the SAME LIQUID, you should be fine. To avoid putting the wrong dropper in the wrong container, only use ONE dropper at a time. That way, you'll always know which container to put it back in!



QUESTIONS

3.A.DATA TABLE

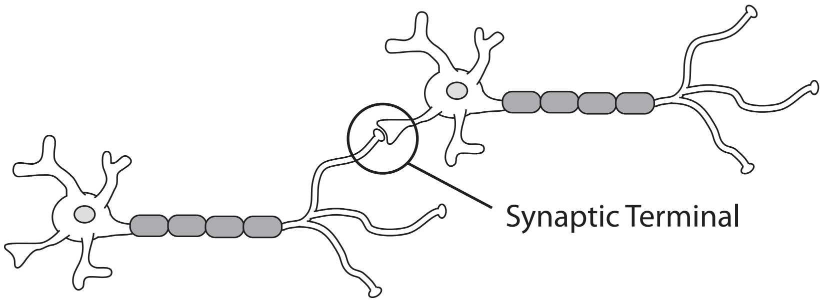
	Normal Sleep (10 hours)	Sleep Restricted (5 hours)
10 minutes		
40 minutes		
100 minutes		

3.A.3) Does the sleep restricted volunteer have normal, increased, or decreased sensitivity to insulin?

3.A.4) What kind of health effects might this have on a person who is regularly getting 5 hours of sleep?

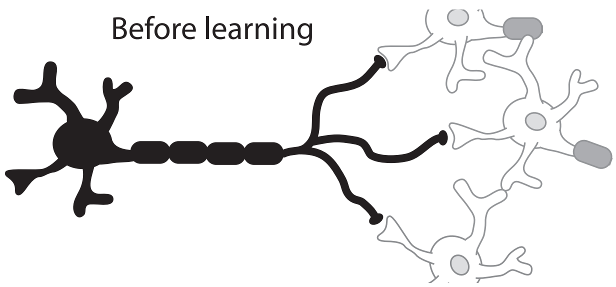
Part 3B: How does sleep affect memory?

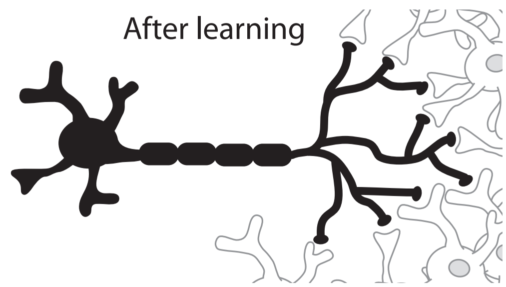
INTRODUCTION



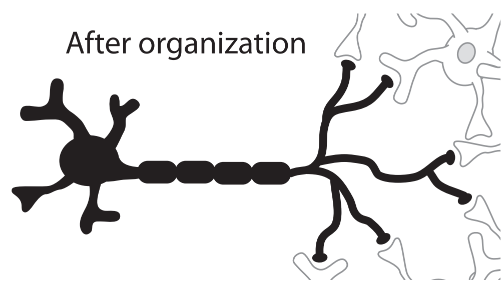
You have learned that neurons communicate with each other at synaptic terminals. The more synaptic terminals there are, the more "synaptic strength" (more electrical signals) there can be.

An animal builds synaptic strength with every experience it has. Whenever you learn something, your neurons can increase the number of branches it has, and therefore increase the number of synaptic terminals.





While it may sound like having more synaptic terminals means you are smarter…that's not really the case. The brain can't keep building an infinite number of synapses – eventually your skull would run out of room! For this reason, the brain is thought to organize synaptic terminals by removing some of them. It's not really "getting rid" of things you learned, it's just making them easier to find.



In a nutshell: Memories have to be stored in a way that doesn't waste a lot of your body's energy. You may make a lot of synapses while you are doing something that is worth remembering, but for you to remember it well, many of those synapses have to get pruned away.

QUESTIONS

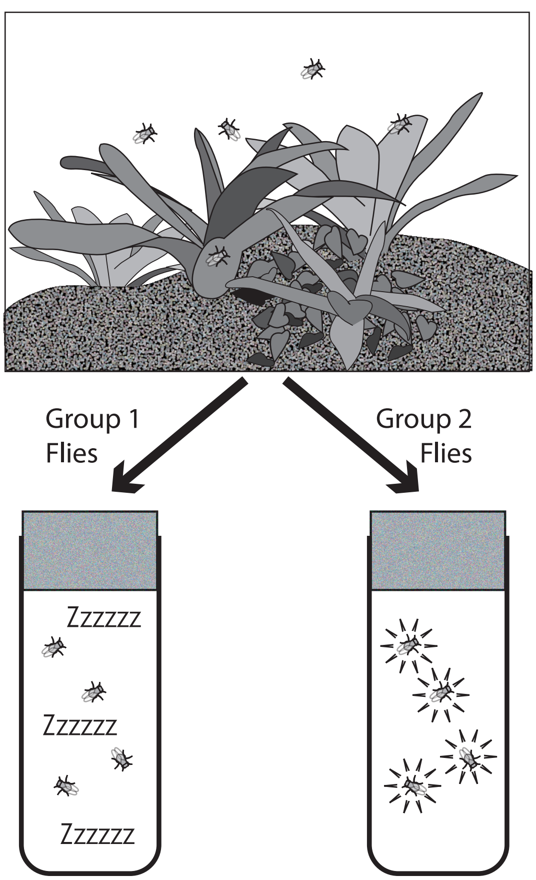
3.B.1) When you learn something, does the number of your synaptic terminals increase or decrease?

3.B.2) What is one way your brain organizes synaptic terminals?

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
12am					
2am					
4am					
6am	Control 1 flies in fly mall	Control 2 flies in boring vials	Group 1 Flies in fly mall	Group 2 Flies in fly mall	
8am					Stain brains for active synaptic terminals
10am					
12pm				Move Group 2 Flies into vials and keep them AWAKE!	
2pm	Dissect and preserve brains	Dissect and preserve brains	Move Group 1 Flies into vials for sleep!		
4pm					
6pm					
8pm			Dissect and preserve brains	Dissect and preserve brains	
10pm					

THE STUDY

Fruit flies are placed into an environment where there are plants, lights, and cool places (for flies) to go. This is called the “fly mall.” One would expect that after spending 12 hours in a fly mall (control 1 flies), the fly brains would have a lot more active synaptic terminals compared to a fly that had been in a boring fly vial (control 2 flies).



In the experiment, these “mall flies” were then either put into a vial and allowed to sleep (Group 1), or put in a vial and kept awake (Group 2).

After each of these conditions, the flies were collected, and their brains were dissected out and preserved, then researchers stained the brains for active synaptic terminals.

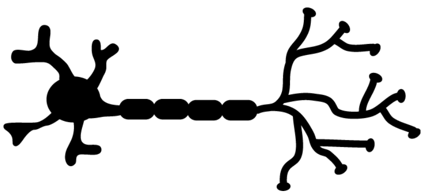
An interesting experience should result in many synaptic terminals being activated. If sleep causes memories to be stored, one should see fewer synaptic terminals after sleep. Or maybe, it’s just that rest causes memories to be stored, in which case, being wide awake in a boring fly vial should do the same thing.

You may have noticed that the poor scientist doing this experiment is basically doing things all day long for a week. This is no made up experiment! Scientists have been known to nap on camp beds in the lab, or split up experiments between two people so that one person isn’t in the lab 24/7 while an experiment like this is going on!

QUESTIONS

3B.HYPOTHESIS
Shown below is a picture of a neuron with lots of synaptic terminals, after learning has occurred. What do you think happens to this neuron when the animal who owns it goes to sleep or doesn't sleep? Draw the neuron shown below, and two other neurons that illustrate your hypothesis: one neuron after the animal has slept. The second picture, of a neuron if the animal has not slept. Will they look the same? Will one have fewer or more synaptic terminals?

After learning (9 synaptic terminals)	After sleeping? (__ synaptic terminals)	After not sleeping? (__ synaptic terminals)



THE EXPERIMENT: Synapse Counting

MATERIALS

• C1 – Control 1: These brain samples are from flies were in the fly mall for 12 hours. They should have a lot of active synapses.

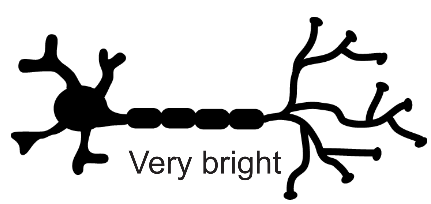
• C2 – Control 2: These flies were in a boring fly vial for 12 hours. They should have very few active synapses.

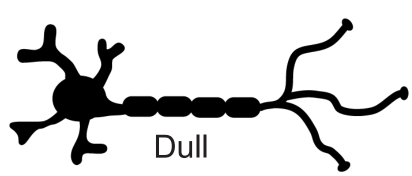
• G1 – Group 1: These brain samples are from flies that were in the fly mall for 12 hours, and then were allowed to sleep for 6 hours in a vial.

• G2 – Group 2: These brain samples are from flies were in the fly mall for 12 hours, and then were kept awake in a vial for 6 hours.

•Black light

You are going to test for the number of synaptic terminals that are active using a fluorescent dye. Flies that have been in different conditions (Described below) had their brains removed and processed into a brain sample. A fluorescent dye is added to the brain sample that detects active synaptic terminals. The more fluorescence you see, the more active synaptic terminals there are on the neurons in the brain sample.





PROTOCOL

The brain samples have already been soaking in fluorescent dye. Your job is to visualize the dye using a black light, and then to record the brightness of the light. A sample with more synaptic terminals will appear bright. A sample with fewer synaptic terminals will appear more dull. We cannot use the brightness to quantify (create a numerical value for) the number of synapses, but we can tell which samples have more and which have less synapses.

1. Put on a pair of safety goggles that have UV protection.

2. Line the four samples up next to each other, and shine a black through them. Describe the amount of fluorescence in each sample (Very bright, bright, or dull?)

Long term exposure to UV light (from a black light, or from the sun) can lead to vision problems like cataracts and macular degeneration.

QUESTIONS

3.B.DATA TABLE

Control 1: Fly mall	Control 2: Vial	Group 1: Fly mall, sleep	Group 2: Fly mall, awake

3.B.3) Did the flies that spent 12 hours in the fly mall have a greater number of active synaptic terminals when compared to flies that spent 12 hours in the vial?

3.B.4) Did the flies that spent 12 hours in the fly mall and then slept (group 1) have fewer active synaptic terminals compared to the ones that were just in the fly mall (control 1)?

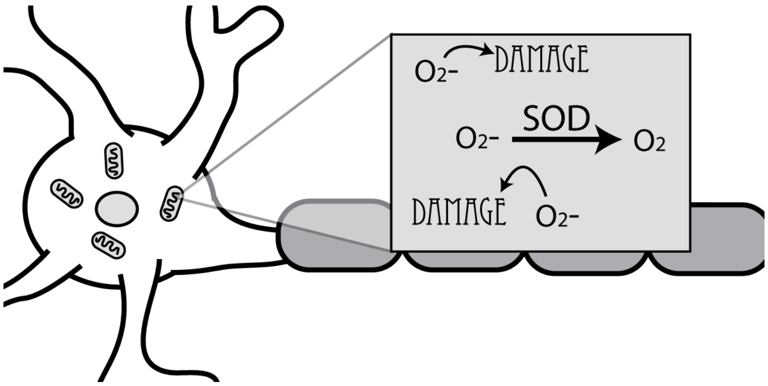
3.B.5) Did the flies that spent 12 hours in the fly mall and then were kept awake (group 2) have fewer active synaptic terminals than the ones that were just in the fly mall (control 1)?

3.B.6) A fly that goes from having a lot of active synaptic terminals during an interesting experience (like a fly mall) to very few active synaptic terminals has probably done a good job of remembering its experience. Is sleep important for a fly to remember an interesting experience?

3.B.7) What kind of health effects might this have on the fly?

Part 3C: Sleep and cellular wastes

INTRODUCTION

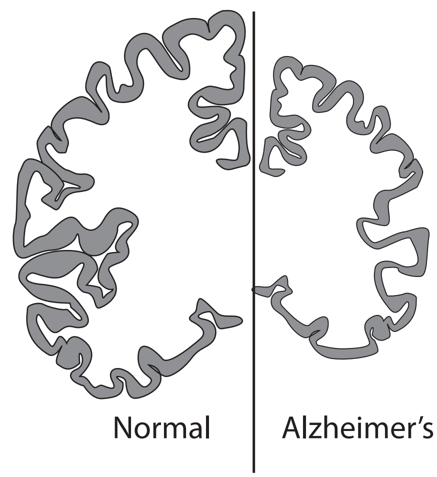


Normal chemical reactions in the cells of your body are constantly creating wastes, particularly when you are awake. One of these wastes is called reactive oxygen species (ROS).

Reactive oxygen species includes the superoxide (O2-) and hydrogen peroxide and are produced mainly in the mitochondria. They're technically not the same as free radicals, but they do the same thing – they steal electrons from other molecules, causing a lot of damage to cells, even causing cell death.

Researchers have done studies that show that ROS that builds up in neurons can be involved in neurodegenerative diseases, like Alzheimer's Disease.

Cross section of normal versus Alzheimer's brain



Our cells get rid of ROS through the activity of enzymes called antioxidants. One of these enzymes is called superoxide dismutase, or SOD for short. SOD turns ROS into harmless chemicals.

In a nutshell: When the body produces a lot of waste, superoxide dismutase has to be active in order to get rid of those wastes.

QUESTIONS

3.C.1) Name a type of waste that is produced by normal chemical reactions in your body.

3.C.2) Name an enzyme that your body uses to get rid of these wastes.

THE STUDY

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Control Mice: Let them sleep for 10 hours a night				*Process brain samples*	
	Experimental Mice: Keep them awake				*Process brain samples*	

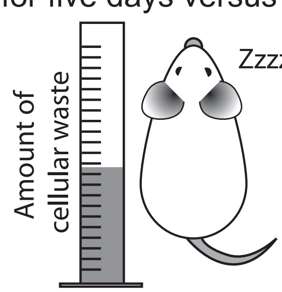
Control mice were allowed to sleep normally for five days. Experimental mice were kept awake for five days. Brain samples from experimental and control mice were processed to detect SOD activity as well as total cellular wastes in the mitochondria of the mice brain cells. The cells came from a region of the brain called the hippocampus. This is a part of the brain that is involved in the consolidation of new memories, emotional responses, navigation and spatial orientation.

QUESTIONS

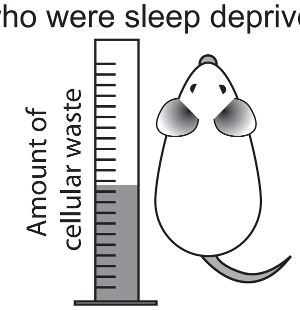
3.C.HYPOTHESIS)
A mouse that is producing a lot of cellular waste should have a lot of SOD activity. Otherwise, those wastes could become toxic to cells. Do you think a mouse that was sleep deprived would produce more or less SOD than a mouse that slept normally? Why?

THE EXPERIMENT: Sleep and Cellular Wastes

Researchers have shown that the total amount of cellular waste in mice that slept normally for five days versus mice who were sleep deprived was the same. This is important to know because you might think that a mouse that did not sleep would produce more metabolic wastes, and would need higher amounts of SOD activity. But it turns out that's not true…a mouse that does not sleep should produce the same amount of SOD as a mouse that slept normally to keep the wastes from causing damage. But is this the case? You will do a titration experiment to find out.

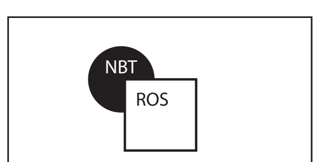


Mouse that slept normally

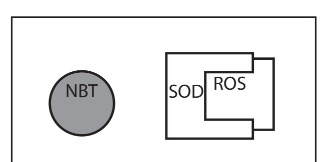


Mouse that did not sleep

Superoxide dismutase can be measured using a chemical called nitro-blue tetrazolium chloride (NBT for short). NBT reacts with ROS to give a deep blue color. Because SOD also reacts with ROS, if SOD is added to NBT+ROS, the SOD will "steal" the ROS and the NBT will appear yellow.

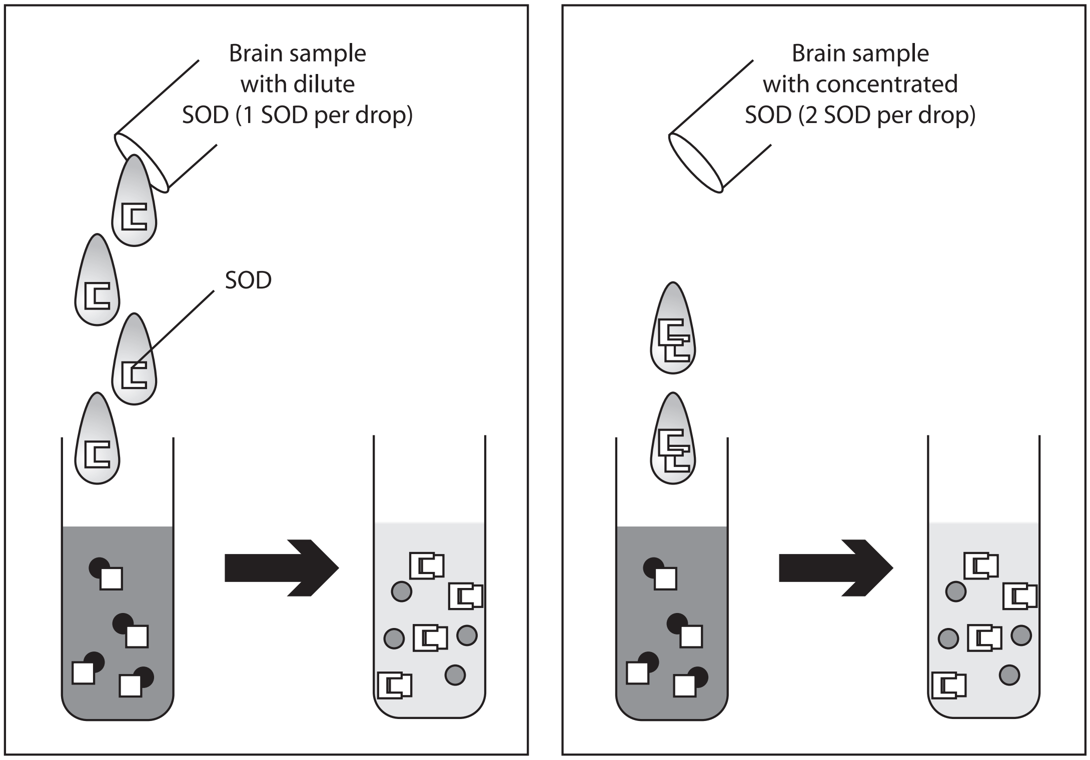


Blue color



Yellow color

If you start with a fixed amount of NBT and ROS in a tube, the liquid will appear deep blue. If you add SOD, the SOD will start to react with ROS and the blue will become less intense until it's almost yellow.



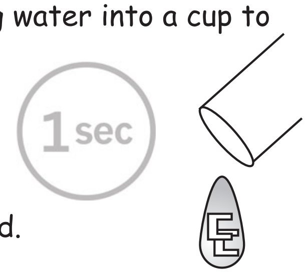
You will measure how much brain sample (containing SOD) you have to add until there are no superoxides that can react with the NBT and the liquid turns yellow. **The more brain protein sample you have to add, the more diluted SOD must be in the brain protein sample.** So if you normally only add 20 drops of SOD to inhibit the NBT and turn the solution yellow, and with your experimental sample you now have to add 10 drops, it means the experimental sample has more SOD per drop than your normal sample.

In a nutshell: The more SOD in your sample, the LESS sample you should need to add.

MATERIALS

- •Two small plastic dishes
- •NBT and ROS solution
- •Control brain sample (normal sleep)
- •Experimental brain sample (no sleep)

This experiment will require that you carefully squeeze a dropper so that one drop comes out at a time. You will be counting drops, so you might want to practice dropping water into a cup to make sure you can squeeze the bulb gently enough to produce about a drop every second.



PROTOCOL

1. Label a small dish “control” and another dish “experimental”

2. Put 1mL of NBT and ROS solution into each of the dishes.

3. Using a clean dropper, count how many drops of control brain sample (normal sleep) you have to add to the control dish to turn the solution yellow. After each drop, swirl the dish and wait about 2 seconds.

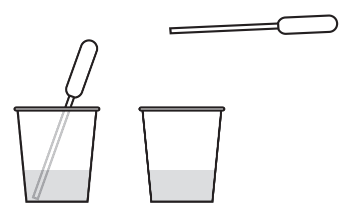
* Every time you drop protein sample into the dish, you want to swirl the dish, and wait about 2 seconds. The solution may swirl into a yellow color, but then as you wait, it will return to bluish green. The solution may also bubble a little, which will make it hard to see the color. Wait for the bubbles to disappear before trying to determine if the solution is really yellow. Take time to wait after EACH drop.

4. Using a clean dropper count how many drops of experimental brain sample (no sleep) you have to add to the experimental dish to turn the solution yellow.

Pay attention, only use one dropper at a time!

In a forensics lab, you would never use the same pipette with more than one liquid. It might mean the difference between being found guilty or innocent!

In an effort to conserve, you may be re-using pipettors. As long as one pipettor is always being used for the SAME LIQUID, you should be fine. To avoid putting the wrong dropper in the wrong container, only use ONE dropper at a time. That way, you’ll always know which container to put it back in!



QUESTIONS

3.C.DATA TABLE

	Control Mice (Normal Sleep)	Experimental Mice (No sleep)
SOD activity (Number of drops of brain sample it took to make the NBT/ROS solution yellow)		

3.C.3) Was there more or less SOD activity in mice that were sleep deprived? (Remember, the more sample you had to add, the LESS SOD activity there is)

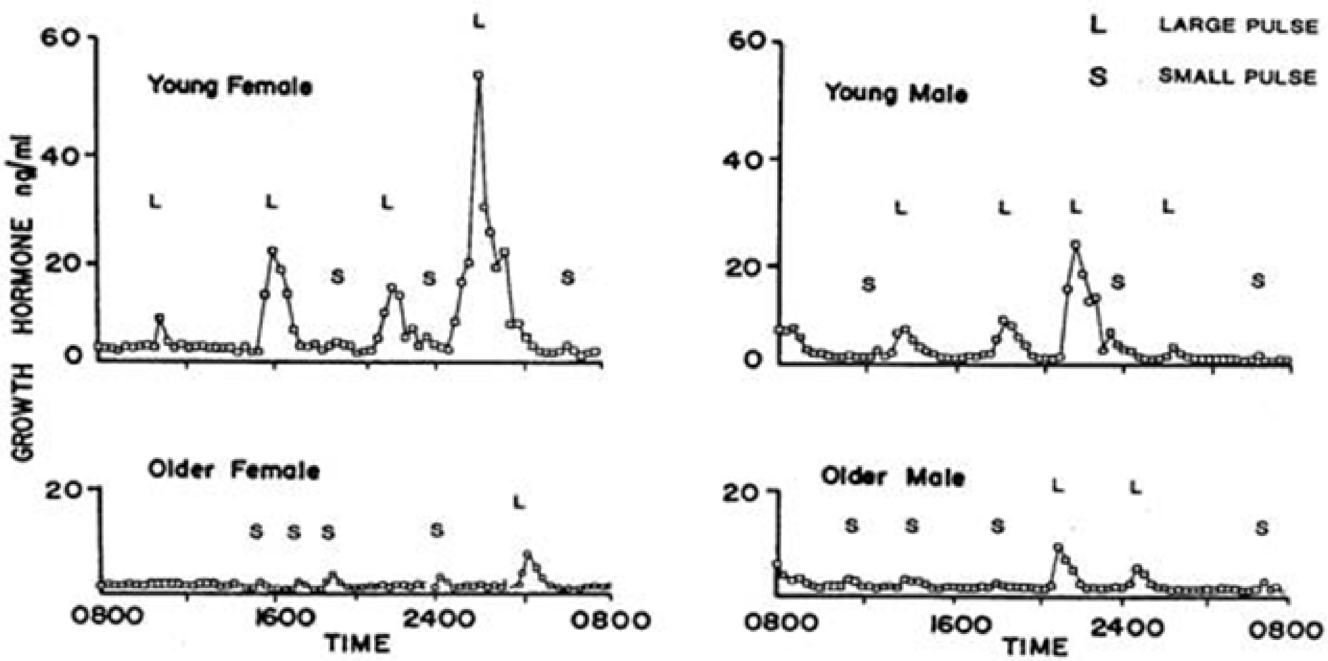
3.C.4) What kind of health effects might this have on the mouse?

PART 3D: Sleep and growth

INTRODUCTION

Growth hormone (GH) is a protein that stimulates growth, cell reproduction and regeneration in humans and other animals. In humans, GH is made and secreted from the anterior pituitary gland in pulses throughout the day. Secretion happens about every 3-5 hours. The biggest pulse of secretion occurs right after you fall asleep. The amount of GH that is secreted depends on a lot of things like age, gender, diet, exercise, stress, and other hormones. Teens secrete the most GH (About 700 micrograms a day) and adults secrete about half that (400 micrograms in a day).

As a child and a teen, GH can make you taller. It also increases muscle mass, strengthens bone, and stimulates the growth of your organs (Except for your brain). When children and teens don't produce enough GH, they may be shorter or smaller than their peers, and may reach sexual maturity later than normal.



This graph shows pulses of GH found in a young versus older woman, and a young versus older man. You can see how big the pulses are for the young male and female versus the older male and female.

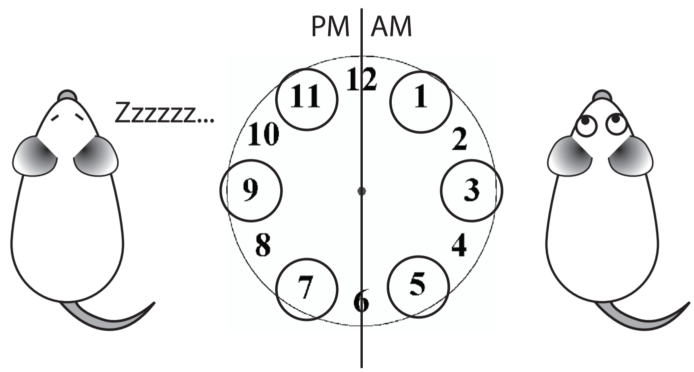
In a nutshell: Growth hormone is secreted in pulses, and it makes you big and strong.

QUESTIONS

3.D.1) Name two things that influence (affect) the amount of Growth Hormone (GH) secreted in a person.

3.D.2) Name one thing that GH does in the human body.

THE STUDY



Researchers examined the secretion of growth hormone in rats that either slept normally, or were deprived of sleep, for seven days. They looked closely at the hourly secretion of growth hormone during nighttime hours, as growth hormone is known to be secreted in spurts rather than at a constant level, all the time. They identified a large spurt of growth hormone that is released about an hour after going to sleep.

QUESTIONS

3.D.HYPOTHESIS
Rats that sleep normally show a large spurt of growth hormone (GH) released about an hour after they fall asleep. What do you think happens during the same nighttime hours for a rat that does not sleep? Would the rat have higher or lower levels of GH at the times shown? Write some possible levels of growth hormone down in the column for the "no sleep" rat that reflect your hypothesis.

	Control (Sleep)	Experimental (No sleep)
7:00pm	0ng/mL GH	
9:00pm	500ng/mL GH	
11:00pm	0ng/mL GH	
1:00am	0ng/mL GH	
3:00am	0ng/mL GH	
5:00am	0ng/mL GH	

THE EXPERIMENT: Growth Hormone and Sleep

You will measure the amount of growth hormone in the six blood samples that were taken from a sleep deprived rat at the same times as when samples were taken from a sleeping rat. You will use an antibody assay to detect the amount of growth hormone present in the blood sample. Red blood cells have been removed from the blood sample, so that it appears clear.

MATERIALS

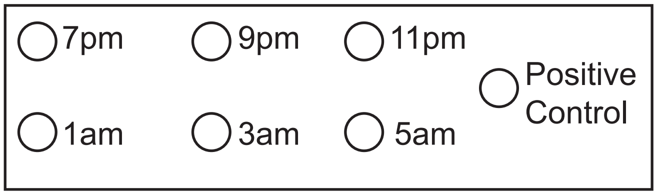
- Blood samples from each time point
  - 7pm
  - 9pm
  - 11pm
  - 1am
  - 3am
  - 5am
- A positive control
- Test paper

It's true, the scientist who did this experiment had to be in the lab from 7pm to 5am. Labs sometimes have camp beds just for these occasions, and alarm clocks so that the scientist remembers to wake up every two hours to take a blood sample!

PROTOCOL

1. Label an antibody card using a pencil as shown below. Each circle should be about 0.5cm in diameter, about the diameter of a pencil eraser.

> To avoid putting the wrong dropper or q-tip in the wrong container, only use ONE thing at a time. That way, you'll always know which container to put it back in!



2. Use a cotton swab to apply the appropriate blood sample and positive control to each circle (put the positive control in the spot labeled "+").

3. Detect the amount of growth hormone present by adding one drop of detection fluid to each circle. Growth hormone will appear as a dark purple color. The darker the color purple, the more growth hormone is present.

4. Use the following color chart to estimate the amount of growth hormone present and record your results in the chart. The positive control contains 300ng/mL of growth hormone.

QUESTIONS

3.D.DATA: Copy the following table, and fill in your results for the sleep deprived rat.

	Control (Sleep)	Experimental (No sleep)
7:00pm	0ng/mL GH	
9:00pm	500ng/mL GH	
11:00pm	0ng/mL GH	
1:00am	0ng/mL GH	
3:00am	0ng/mL GH	
5:00am	0ng/mL GH	

Did your positive control work?_______________

3.D.3) Did the sleep deprived rat produce the same, more, or less growth hormone than the rat that slept normally?

3.D.4) What kind of health effects might this have on the rat?

PART 4 What are people saying about YOU and sleep?

See what the Mayo Clinic has to say about teens and sleep...

Teens are notorious for staying up late and being hard to awaken in the morning. If your teen is no exception, it's not necessarily because he or she is pushing the limits or fighting the rules. This behavior pattern actually has a physical cause — and can be modified to improve your teen's sleep schedule.

A TEEN'S INTERNAL CLOCK
Everyone has an internal clock that influences body temperature, sleep cycles, appetite and hormonal changes. The biological and psychological processes that follow the cycle of this 24-hour internal clock are called circadian rhythms. Before adolescence, these circadian rhythms direct most children to naturally fall asleep around 8 or 9 p.m. But puberty changes a teen's internal clock, delaying the time he or she starts feeling sleepy — often until 11 p.m. or later. Staying up late to study or socialize can disrupt a teen's internal clock even more.

TOO LITTLE SLEEP
Most teens need about nine hours of sleep a night — and sometimes more — to maintain optimal daytime alertness. But few teens actually get that much sleep regularly, thanks to factors such as part-time jobs, early-morning classes, homework, extracurricular activities, social demands, and use of computers and other electronic gadgets. More than 90 percent of teens in a recent study published in the Journal of School Health reported sleeping less than the recommended nine hours a night. In the same study, 10 percent of teens reported sleeping less than six hours a night.

Although this might seem like no big deal, sleep deprivation can have serious consequences. Tired teens can find it difficult to concentrate and learn, or even stay awake in class. Too little sleep also might contribute to mood swings and behavioral problems. Another major concern is drowsy driving, which can lead to serious — even deadly — accidents.

> Adjust the lighting. As bedtime approaches, dim the lights. Then turn off the lights during sleep. In the morning, expose your teen to bright light. These simple cues can help signal when it's time to sleep and when it's time to wake up.
>
> Stick to a schedule. Tough as it may be, encourage your teen to go to bed and get up at the same time every day — even on weekends. Prioritize extracurricular activities and curb late-night social time as needed. If your teen has a job, limit working hours to no more than 16 to 20 hours a week.
>
> Nix long naps. If your teen is drowsy during the day, a 30-minute nap after school might be refreshing. Be cautious, though. Too much daytime shut-eye might only make it harder to fall asleep at night.
>
> Curb the caffeine. A jolt of caffeine might help your teen stay awake during class, but the effects are fleeting — and too much caffeine can interfere with a good night's sleep.
>
> Keep it calm. Encourage your teen to wind down at night with a warm shower, a book or other relaxing activities. Discourage stimulating activities — including vigorous exercise, loud music, video games, television, computer use and text messaging — an hour or two before bedtime.

Know when to unplug. Take the TV out of your teen's room, or keep it off at night. The same goes for your teen's cellphone, computer and other electronic gadgets.

Sleeping pills and other medications generally aren't recommended. For many teens, lifestyle changes can effectively improve sleep.

IS IT SOMETHING ELSE?
In some cases, excessive daytime sleepiness can be a sign of something more than a problem with your teen's internal clock. Other problems can include:

Medication side effects. Many medications — including over-the-counter cold and allergy medications and prescription medications to treat depression and attention-deficit/hyperactivity disorder — can disrupt sleep.

Insomnia or biological clock disturbance. If your teen has trouble falling asleep or staying asleep, he or she is likely to struggle with daytime sleepiness.

Depression. Sleeping too much or too little is a common sign of depression.

Obstructive sleep apnea. When throat muscles fall slack during sleep, they stop air from moving freely through the nose and windpipe. This can interfere with breathing and disrupt sleep. You might notice loud snoring or intermittent pauses in breathing, often followed by snorting and more snoring.

If you're concerned about your teen's daytime sleepiness or sleep habits, contact his or her doctor. If your teen is depressed or has a sleep disorder, proper treatment can be the key to a good night's sleep.

QUESTIONS

4.1) How might not getting enough sleep affect your metabolism of sugar?

4.2) How might not getting enough sleep affect your ability to learn new things?

4.3) How might not getting enough sleep affect the amount of toxic cellular wastes in your body?

4.4) How might not getting enough sleep affect a person's growth?

4.5) Think out of the box: Researchers aren't yet sure whether or not short sleepers suffer any of the health effects that people do, when they don't get enough sleep…but if they do NOT, why do you think they don't? What do you think makes them different?

4.6) How much sleep does the Mayo Clinic think you should have?

4.7) What do you think is "Enough sleep?"

Now let's go back to some of the questions we answered before and see if your answers have changed…

4.8) Are you interested in training yourself to sleep less?

4.9) About how much sleep do you get on weeknights?

4.10) About how much sleep do you get on weekends and holidays?

4.11) Do you think this is "Enough sleep?"

4.12) Do you think there are any health effects to not getting "Enough sleep?"

4.13) Did any of your answer change? Why?

4.14) If you feel you are not getting enough sleep, describe 2-3 things you could do to get more sleep.

careers and science

Sleep Lab Technician

A career in sleep is as intriguing as it sounds. How exactly does one fall into – no pun intended – a career of sleep? What are sleep labs and what do they test for in there? Renee D. Short was a full-time nursing student when she started working in an adult sleep lab. She decided to stay in the field after she was done with school and eventually left the adult lab for the pediatric sleep medicine service, so she can work with children.What a sleep study does is monitor people in their sleep to see if they are having problems sleeping that they may never know about, since they are unconscious when it happens!

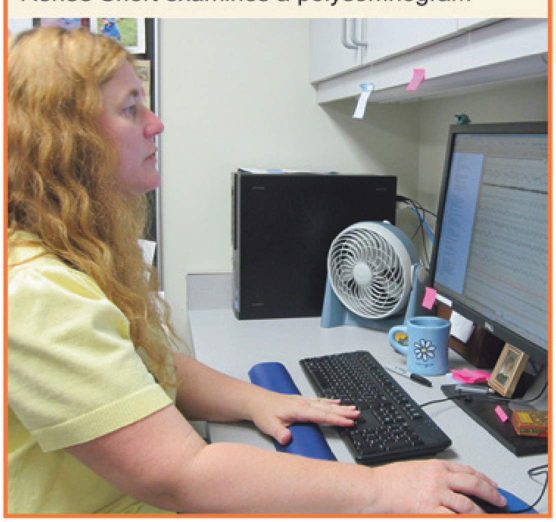

A young patient is prepared for a polysomnogram (photo by Robert Lawton)

Polysomnograms are used to diagnose many types of sleep disorders. They typically requirie about 22 wire attachments to the patient. All together, these will measure EEG (impulses between neurons), airflow, chin muscle tone, leg movements, eye movements, heart rate and rhythm, oxygen saturation, chest wall movement, and upper abdominal wall movement.

The main difference between an adult and a pediatric sleep lab is that the latter has to be a lot more accommodating, so the children will feel comfortable enough to spend the night there. The reception area in Golisano Children's Hospital Pediatric Sleep Medicine Services has bright-colored walls, a train track and handprint art on the walls.

Though it takes more effort to convince a child to wear sensors, electrodes and tubes to bed, particularly when they have children with special needs in the lab, Renee does not mind spending a little more time to desensitize the child. She usually puts on a DVD to distract them or gives them smiley face stickers that pretend to be sensors, before putting the real thing on them. There are also two beds in the "sleep rooms" – one for the child and the other for the parent. When they finally do go to sleep, they are monitored for rapid eye movements (REM), brain activity, airflow, heart rate, chin tenacity, oxygen saturation levels and more.

Renee Short examines a polysomnogram



As the registered polysomnographic technologist, Renee prepares a two-page report for the parents on how well their child is sleeping. A common sleep disorder among children is sleep apnea, characterized by abnormal pauses in breathing. This disrupts their sleep and leaves them feeling tired the day after, even after a night of sleep!

Entry-level jobs are available for high school graduates with 6 months of direct patient care experience or 1 year of post-secondary education. Moving up a level requires successful completion of a polysomnography program in no less than a year, or more direct experience as a sleep trainee.
Median expected salary: $48,077