Caffeine and Neural Fatigue

Core Concepts

Activation of the brain and nerve cells results in activation of muscles. This process can break down at different places, resulting in fatigue. Receptors send signals within a cell to cause cellular events to happen. Receptors can be bound by substances that act as activators or inhibitors.

Supplies You'll Need

For 30 students working in groups of 2, with each experiment set up as a station with 15 students working at each station at any one time. This set up should be sufficient for 2 consecutive classes of 30 students.

48 3-oz plastic bathroom cups
16 small plastic trays or plates (ex: ½ Petri dish)
16 pairs of forceps
45 droppers
Chromatography paper

1 gram Luminol powder
100mL 2.0 M Sodium Hydroxide
125mL 3% Hydrogen Peroxide
20mL Bleach
200mL White Vinegar
150mL 0.1% Bromothymol Blue

Class Time Required

Approximately three 60 minute class periods.

Solutions You'll Need to Make

Luminol and Hydrogen Peroxide Solution
**This solution can be prepared then stored in the refrigerator for at least two months

Step 1: Luminol in base:
Mix 100mL of 2M NaOH solution with 25mL water. Add 1g of luminol powder, mix well. This should produce a yellowish solution. This solution is highly caustic, so be careful! This solution can be stored for 6 months in a heavy duty plastic bottle (base will etch glass bottles)

Step 2: Luminol/Peroxide solution:
Add 125mL 3% hydrogen peroxide solution to above luminol in base solution. This will result in a 50/50 solution of Luminol in base and 3% hydrogen peroxide. This solution should be prepared at least a day in advance and stored in the refrigerator for a bright result. This is recommended as it seems to give better results when it's cold.

10% Bleach Solution
180mL water
20mL bleach

Ordering Information (From Wards)

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

Luminol powder
#9459801 1g bottle $9.10

2.0M Sodium Hydroxide
#9708207 1L bottle $6.75

0.1% Bromothymol Blue
#9447106 500mL bottle $11.30

SAFETY CONCERNS

Luminol is only soluble in a solution of about 6.4% NaOH. This is about a 1.6M solution of NaOH. The luminol solution that students will have has a final concentration of 3.2% NaOH, or about 0.8M NaOH. MSDS sheets indicate the following safety measure for 0.1-2M solutions of NaOH:

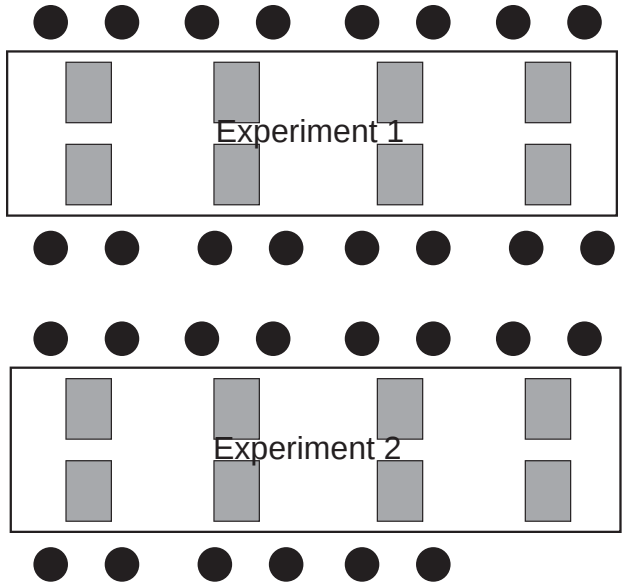
Emergency and First Aid Procedures:
- Eyes - In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Skin - After contact with skin, wash immediately with plenty of water.
- Ingestion - If swallowed, if conscious, give plenty of water and induce vomiting immediately as directed by medical personnel. Immediately call a physician or poison control center. Never give anything by mouth to an unconscious person.

Hydrogen peroxide, bleach (sodium hypochlorite), and white vinegar can all be bought at any supermarket.

Laboratory Set-up

There are two experiments in this lesson. Rather than set up both experiments for each group of two students, we recommend setting each experiment up as a station with eight "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 two stations. Students can rotate to complete both experiments

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



Experiment 1: Adenosine Receptor Activation Assay

3 3oz plastic cups labeled and filled as follows:

- o"Adenosine" = 10mL 10% bleach
- o"Caffeine" = 10mL 100% white vinegar
- o"AR Cell Culture" = 10mL Luminol/Peroxide Solution

Place a plastic dropper into the Adenosine and Caffeine cups

Place at least 4 (2 per group using the station) 0.5x0.5inch squares of chromatography paper in the AR Cell Culture solution

Place one pair of forceps and a single plastic dish on the placemat

Experiment 2: Ryanodine Receptor Activation Assay

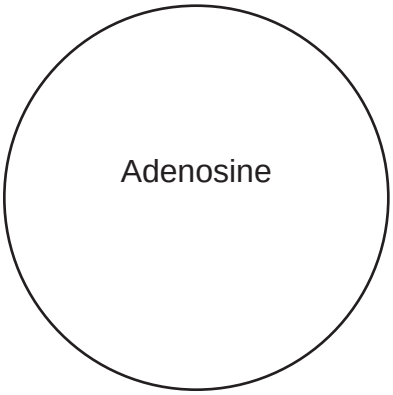
3 3oz plastic cups labeled and filled as follows:

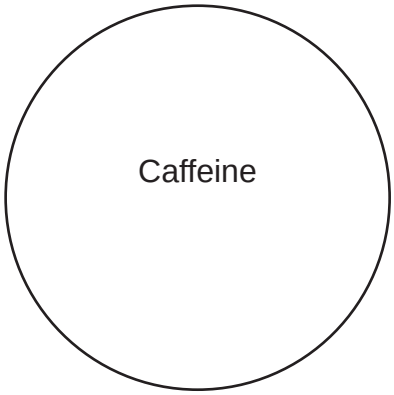
- o"Ryanodine" = 10mL 100% white vinegar
- o"Caffeine" = 10mL 100% white vinegar
- o"IR Cell Culture" = 0.1% Bromothymol Blue

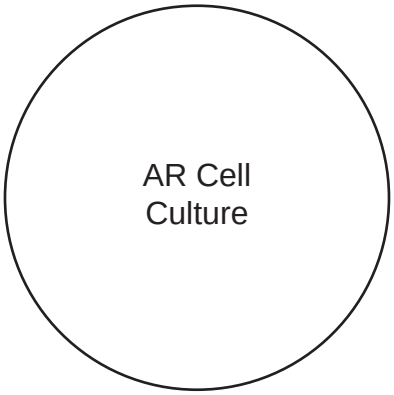
Place a plastic dropper into the Ryanodine and Caffeine cups

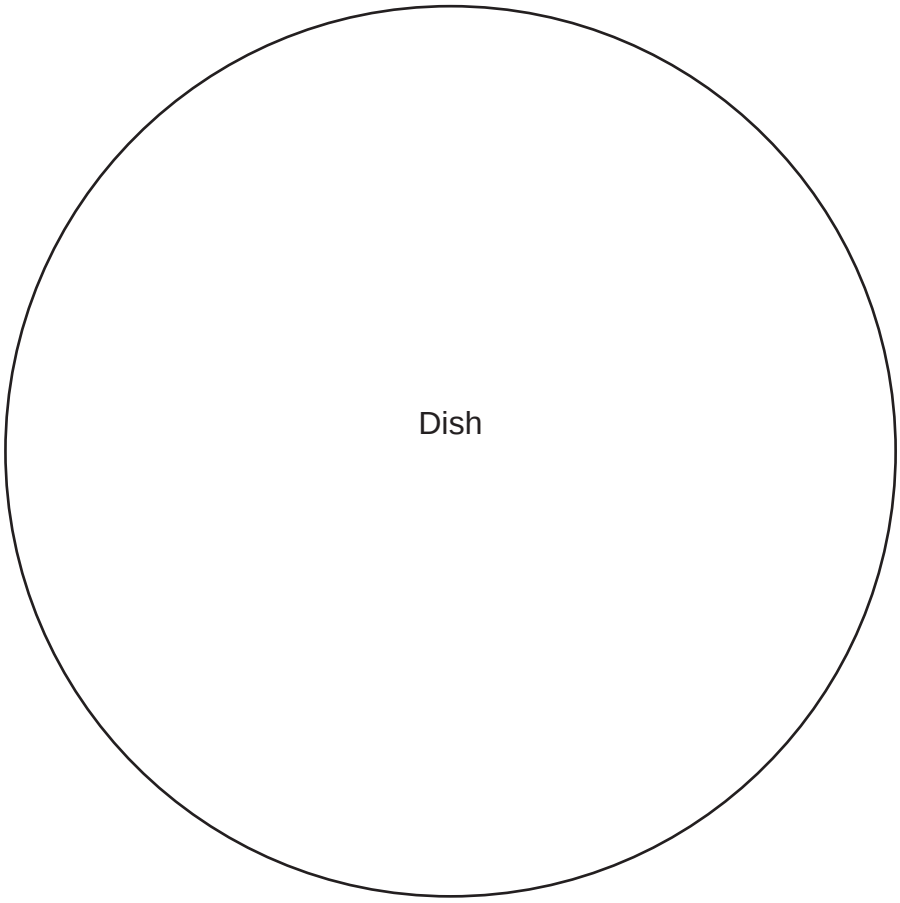
Place at least 4 (2 per group using the station) 0.5x0.5inch squares of chromatography paper in the IR Cell Culture solution

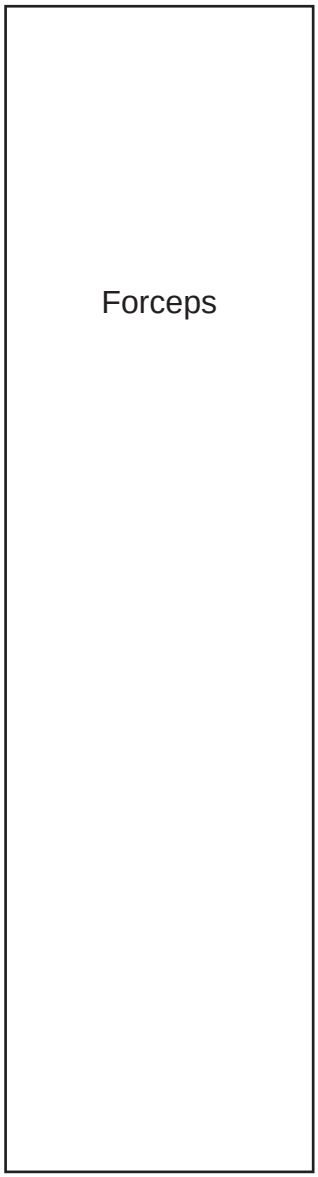
Place one pair of forceps and a single plastic dish on the placemat





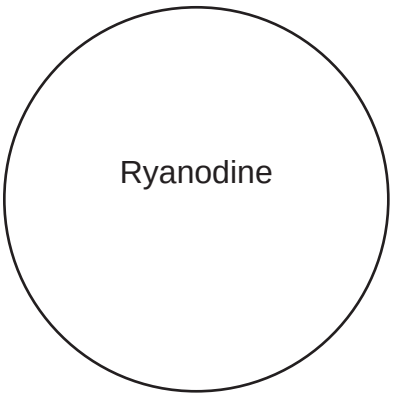


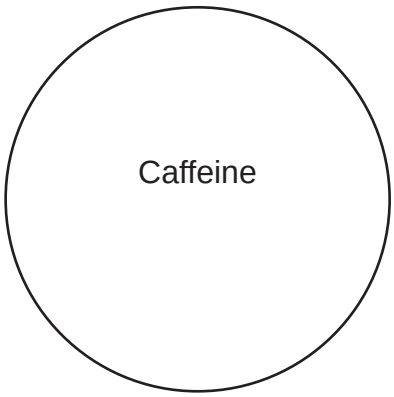


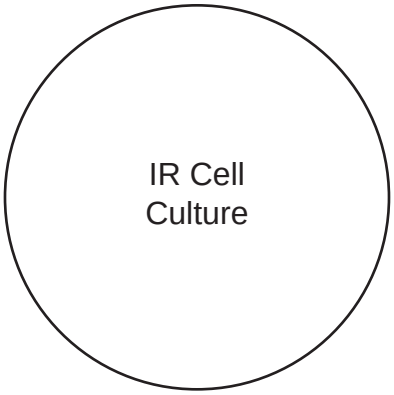


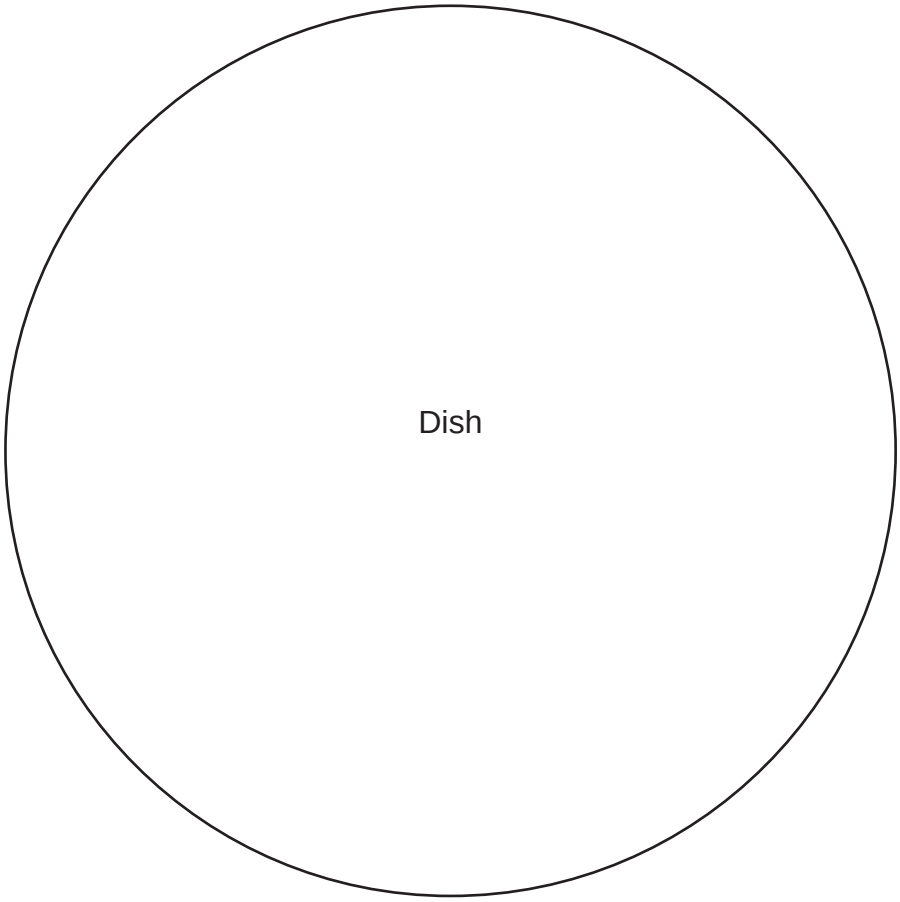
Adenosine Receptor Activation Assay Placemat

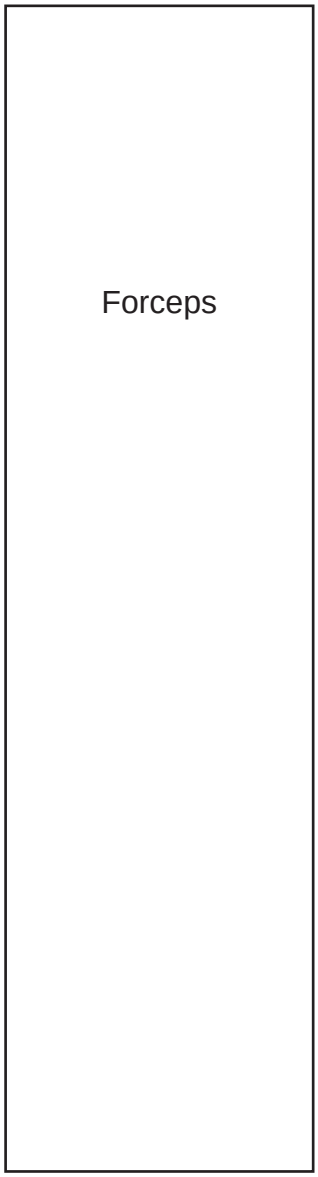
Follow all the directions in your hand-out for how to perform this experiment. Keep all droppers in their proper containers, do NOT mix droppers. When you are done, rinse off your dish with water, and dry it so that other students can use this station.











Ion Receptor Activation Assay 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. When you are done, rinse off your dish with water, and dry it so that other students can use this station.

Suggested Class Procedure

Before class period 1

1. Make copies of the student booklet. These can be reused for multiple classes and is meant to be printed out double-sided, and hole-punched for a folder cover. Students should print answers to questions in their own lab notebook (using lined paper or a spiral notebook), or print out one copy of the student answer sheet per student (this is provided as a Word document so you can change the questions if you want to).
2. Students should have some background in neuron structure and function in order to complete these activities.
3. Distribute a copy of the student booklet. Ask students not to read ahead.
4. Have students read pages 2-3 for homework and answer questions, as an introduction to the activity.

Class period 1

5. Go through answers to questions from pages 2-3.
6. Have students read page 4 “Coffee before the big race.” Ask students to comment on whether or not they think coffee would help an athlete. Students may have some experience with coffee or caffeinated drinks (Red Bull, Coca Cola) making them sleepy, or perking them up, or making them jittery. Focus students on how caffeine might interact with the receptors they will be discussing, the adenosine and ion receptors. If they knew whether caffeine activated or inhibited the receptors, could they make a better case for whether or not caffeine should be considered a performance enhancing drug, and perhaps banned from athletic events?
7. Have students read part 3 and answer questions 3.1-3.3 to make sure they understand the Adenosine Receptor Activation Assay Lab. Have them prepare a graphical flow chart for the experiment, with spaces to answer questions 3.4-3.6 (See student answer sheet for a sample).
8. Have students read part 4 and answer questions 4.1-4.4 to make sure they understand the Ion Receptor Activation Assay Lab. Have them prepare a graphical flow chart for the experiment, with spaces to answer questions 4.4-4.6 (See student answer sheet for a sample).

Class period 2

9. Allow students to perform the lab and answer questions 3.4-3.8 to record their results and make conclusions.
10. Allow students to perform the lab and answer questions 4.4-4.8 to record their results and make conclusions.
11. Have a discussion about whether or not this data is enough to result in the ban of coffee or caffeine consumption before athletic events.

Class Period 3 (This part may require some extra time for students who do not have a lot of experience examining a table of scientific results)

12. Read page 11. This part raises the question of whether or not knowing that something works in a test tube or a model organism really means that it works on people. Students will be asked to analyze data from a real review paper, using a step by step process outlined in the booklet.
13. Have students look at the table, and see what they make of it. Try to use it to answer the question of whether or not caffeine can improve athletic performance.
14. Have students read the rest of the case, where the table is simplified for two examples. Have them fill in the rest of the table. If you are concerned about the difficulty, a filled in table is provided as a separate pdf document, or you can fill it in as a class using the information in the answer key.
15. Answer questions for part 5.

Career Articles are attached to the end of the student handout for students to look at on their own time. These are not necessarily careers IN science, but careers (or in this case, sports) where individuals have some knowledge of science that helps them do what they do...better. Optional discussion questions include, “Are you interested in this career? Why/why not?” or “How, specifically, does this career tie in with neuroscience and the activity we just completed?”

Sample Student Lab Notebook (Answer Key)

Part 1

1.1) During muscle fatigue, the muscle isn't working properly.

1.2) During neural fatigue, the brain, the upper motor neuron, or the lower motor neuron aren't working properly

1.3) A neuromodulator can control the amount of neurotransmitter a neuron releases.

1.4) When an ion receptor is activated it causes the release of more neurotransmitter

1.5) When an adenosine receptor is activated it causes the release of less neurotransmitter

Part 2

2.1) The coaches are concerned about students drinking coffee/ caffeine before a track meet. They think this might give students who drink coffee an unfair advantage over students who do not.

2.2) Marion wants to know if caffeine just makes you less sleepy or if it gives you an unfair advantage.

2.3) You would need to know if caffeine can really make someone a faster runner or a stronger jumper.

2.4) Caffeine makes my hyper, I think I could run faster if I drank coffee or a Red Bull.

2.5) I think caffeine acts as an inhibitor of the adenosine receptor, because adenosine makes you tired and caffeine makes you hyper.

2.6) I think caffeine acts as an activator of the ion receptor, because when ion receptors are activated, you can send signals better, and you'd be faster and stronger.

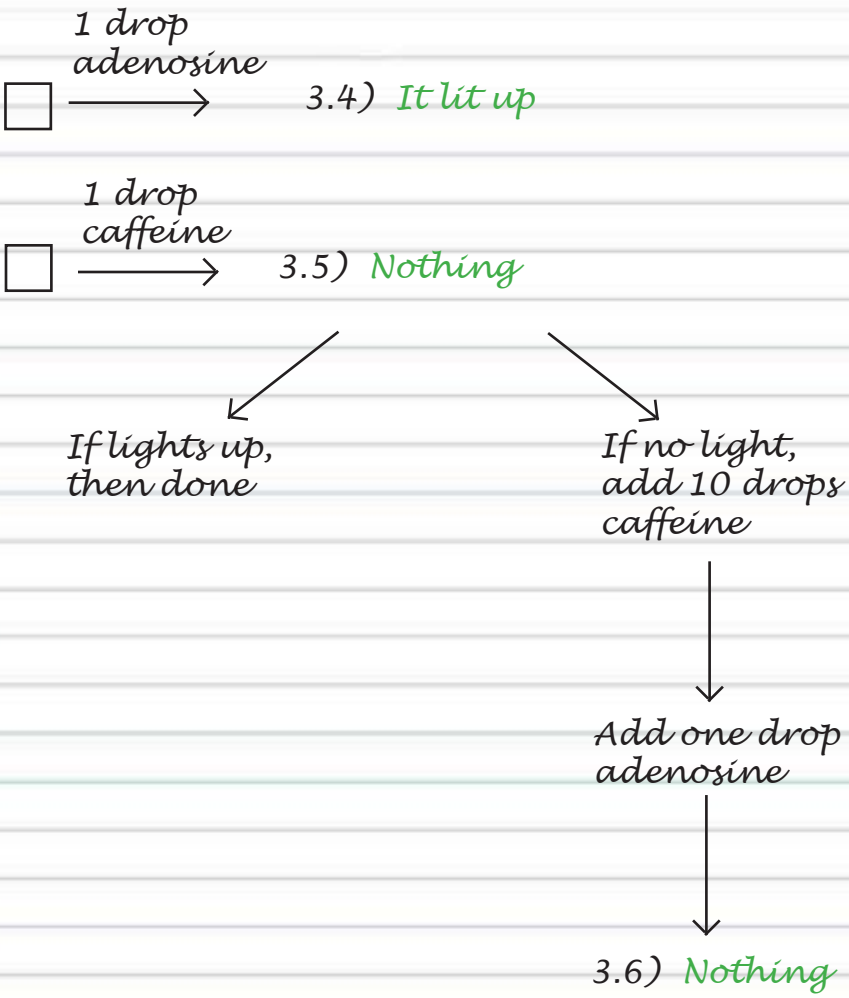
Part 3

3.1) If you add adenosine to the AR cell culture, I think I would you would see a flash of light because adenosine is an activator of the adenosine receptor.

3.2) If caffeine was an inhibitor, I would not see a flash of light if I added caffeine to the AR cells, because an inhibitor does not activate the receptor.

3.3) If caffeine was an activator, I would expect it to act the same way as adenosine so I would see a flash of light.

Flow Chart



3.7) Caffeine is an inhibitor of the adenosine receptor. I say this because it prevents adenosine from causing the light reaction.

3.8) Yes, caffeine could be acting as a performance enhancer, because it makes you less tired and makes your neurons secrete more neurotransmitter.

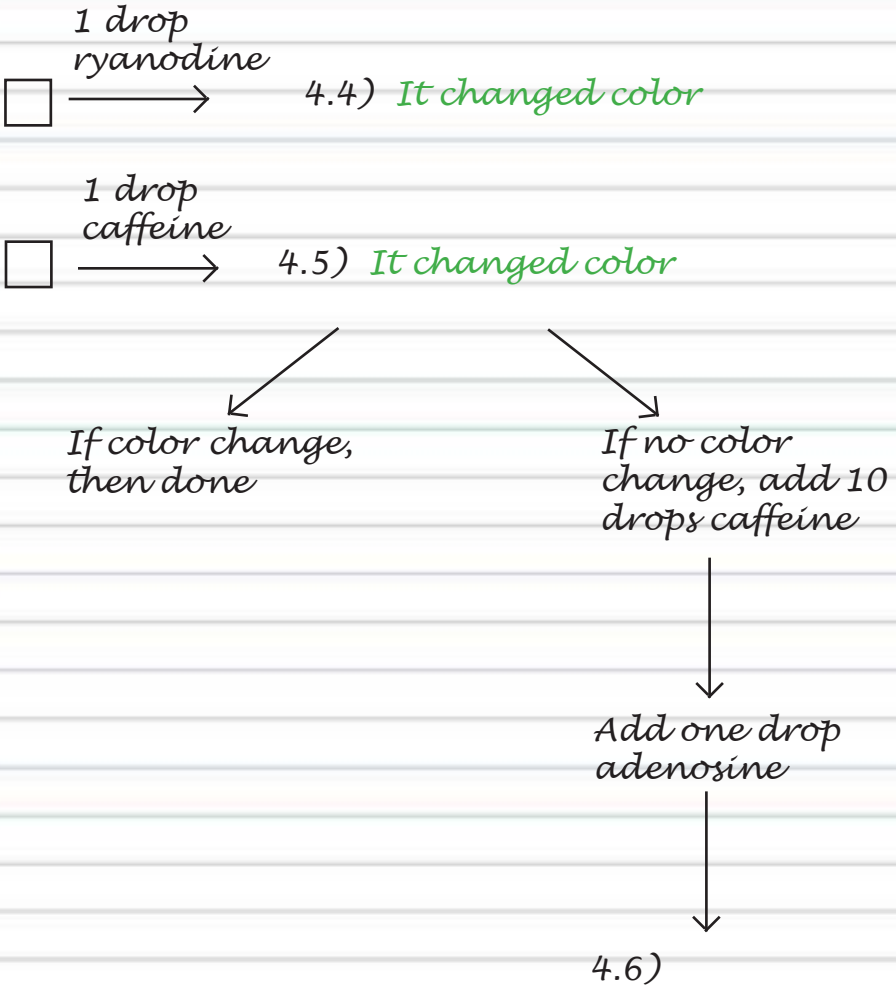
Part 4

4.1) If I add ryanodine to the IR cells, I would see a color change.

4.2) If caffeine were an activator of the ion receptor, I should see a color change if I add caffeine to the IR cells.

4.3) If caffeine were an inhibitor of the ion receptor, I should see no color change if I add caffeine to the IR cells.

Flow Chart



4.7) Caffeine is an activator of the ion receptor.

4.8) Yes, caffeine could be acting as a performance enhancer, because it makes your neurons secrete more neurotransmitter.

Part 5

5.T:

Reference (author of study)	Are participants specialists in any sports?	What did the participants do?	What was measured in the key results?	Was the result significant?
Ivy	Trained cyclists	Cycle for 2 hours	Work and amount of fat oxidized	No
Cohen	Trained runners	Run 21km (hot and humid)	Time	No
Berglund	Trained skiers	Raced about 20km	Time	Yes
MacIntosh	Trained swimmers	Swim 1500m	Time	Yes
Wemple	Not trained, just active	Worked out for 3 hours	Time	No
Collomp	Trained swimmers and untrained swimmers	Swim 200m	Time	Trained: Yes Untrained: No
Collomp	Not trained, just active	Cycled "all out" for 30 sec	Power	No
Anselme	Not trained, just active	Sprinted for 6 seconds for a few times	Power	Yes

**You may wish to provide this table to the students, or do this together, if your students haven't had a lot of experience with reading data tables.*

5.1) This table is a summary of studies that look at the effects of caffeine on athletic performance.

5.2) Eight

5.3) If you are measuring time or speed, then a trained athlete does better with caffeine. If you are measuring work done, or amount of fat oxidized, it doesn't do anything.

5.4) If you are measuring time or speed, then an active individual doesn't do better with caffeine. If you are measuring power, sometimes they do better, sometimes they don't.

5.5) Answers may vary

5.6) Answers may vary

Bibliography

T.E. Graham, E. Hibbert, P. Sathasivam. (1998) Metabolic and exercise endurance effects of coffee and caffeine ingestion. Journal of American Physiology, 85:883-889

This paper describes an experiment done, in which, the researchers gave test subjects caffeine in coffee or dissolved in water. They found that although the amount of caffeine found in plasma was the same across all test subjects, the amount of epinephrine in plasma was much greater when subjects ingested caffeine versus coffee. They concluded that there must be a component of coffee that moderates the action of caffeine in the body.

T.E. Graham. (2001) Caffeine and Exercise, Metabolism, Endurance and Performance. Journal of Sports Medicine, 31(11): 785-807

This paper is a review that explores whether or not caffeine can be considered a performance enhancing drug and reviews how caffeine might be acting to confer greater athletic performance. It is not actually known exactly how caffeine can produce gains in endurance and athletic performance, although its action upon adenosine receptors and ryanodine receptors (both in neurons and in muscle) must play some role. Dr. Graham makes the case in this paper for the effects of caffeine on creating a favorable intracellular ionic environment (such as increased calcium released by ryanodine receptors embedded in the sarcoplasmic reticulum) in muscle cells as a way of increasing the amount of force produced in the muscle. He also makes the case against using caffeine as a performance enhancer, even though it is legal to do so. As he says “Athletes who ingest caffeine are using a drug for the express purpose of gaining an advantage. As such, the author considers it to be doping and unethical. If an athlete has made a conscious decision to take caffeine for the purpose of gaining an advantage and enhancing performance, this could be the first of a series of similar decisions for other drugs.”

K. B. Hansen, P. Mullasseril, S. Dawit, N. L. Kurtkaya, H. Yuan, K. M. Vance, A. G. Orr, T. Kvist, K. K. Ogden, Phuong Le, K. M. Vellano, I. Lewis, S. Kurtkaya, Y. Du, M. Qui, T. J. Murphy, J. P. Snyder, H. Bräuner-Osborne, S. F. Traynelis. (2010) Implementation of a Fluorescence-Based Screening Assay Identifies Histamine H3 Receptor Antagonists Clobenpropit and Iodophenpropit as Subunit-Selective N-Methyl-d-Aspartate Receptor Antagonists. Journal of Pharmacology and Experimental Therapeutics, 333(3):650-662

This paper describes a receptor activation assay that was used to create the mock ryanodine receptor activation assay. In this paper, the authors created an assay to detect activation of the NMDA receptor, a ligand gated ion channel. A change in calcium results in a change in fluorescence that is detected by a fluorescence microscope.

(viewed on 1.31.12). The Synapse, CNS CLINIC, http://www.humanneurophysiology.com/index.htm

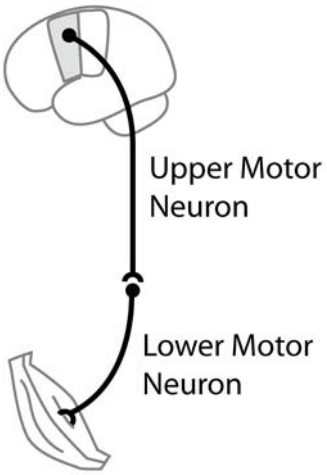
This website contains multiple pages related to the central nervous system. The synapse page describes impulse conduction at a synapse. The key ideas taken from this website are the factors affecting synaptic transmission, specifically, that calcium concentration plays a role in how much neurotransmitter is released, and that a neuron integrates information from hundreds to thousands of synapses with input neurons.

J.M. Kalmar. (2005) The influence of caffeine on voluntary muscle activation, Medicine and science in sports exercise, 37(12):2113-2119

This paper reviews the effects, of caffeine on voluntary muscle activation, that have been demonstrated in a number of other studies. It suggest that caffeine can affect processes all along the pathway from the brain to the muscle, from the ability to activate a movement in the brain, to the ability of the upper motor neuron to send impulses and the ability of the lower motor neuron to receive them, to the ability of the muscle to respond. . Which one is the most important to caffeine’s role as a performance enhancer is still unknown.

Caffeine and Neural Fatigue

PART 1: Introduction to Neural Fatigue



The Difference Between Muscle and Neural Fatigue: You have learned that nerve cells communicate with each other through electrical and chemical signals. You also know that for voluntary muscle movement, impulses need to travel through neurons from the brain to the muscles. Whenever you move a muscle you are involving at least two motor neurons, an upper motor neuron and a lower motor neuron.

So is it true that whenever your brain "thinks" to move your muscles, it always happens? No, not really. You've probably been asked to run a few miles in your PE class, or do a bunch of sit-ups. Eventually, you might have gotten to a point of "fatigue." You were so tired that, even though you wanted or needed to keep going, your body just couldn't do it. Maybe you walked instead of ran, or just lay there while other people kept doing sit-ups.

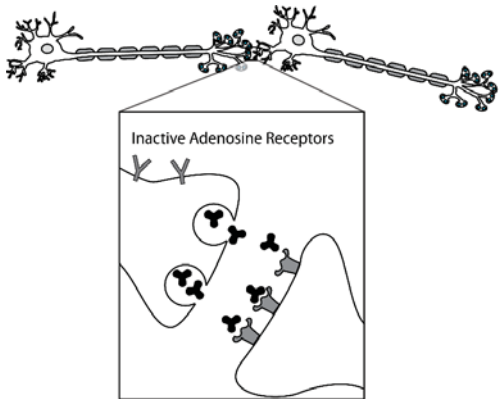
There are two body systems that are affected when you feel fatigued, the muscular and the nervous systems. Most people know about muscle fatigue. Your muscles just can't contract anymore, no matter how many signals your neurons send. Some researchers have said that this is caused by the build-up of lactic acid. You can also get neural fatigue. In this case, your muscles are ready to move, but either your brain isn't sending a signal, or the upper motor neurons are getting a signal from the brain, but they can't activate the lower motor neuron.

QUESTIONS

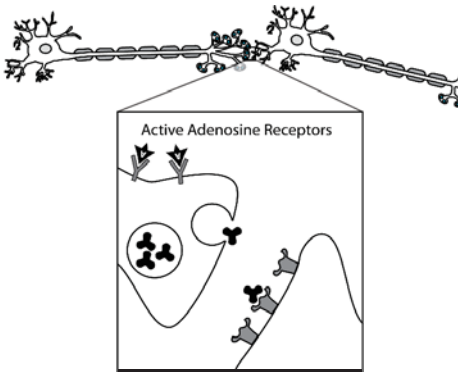
1.1) When you are suffering from muscle fatigue, which of the following parts isn't working? The brain, the upper motor neuron, the lower motor neuron or the muscle?

1.2) When you are suffering from neural fatigue, which of the following parts isn't working? The brain, the upper motor neuron, the lower motor neuron or the muscle?

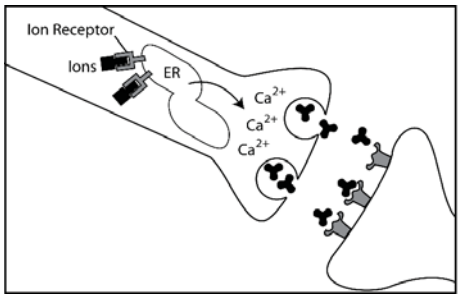
How You Get Neural Fatigue: There are two ways you can get neural fatigue. One involves the chemical adenosine. The other involves changing concentrations of ions in the upper motor neuron.



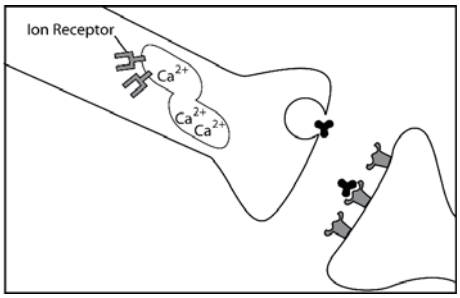
Your brain has receptors for the chemical adenosine. When these receptors are NOT activated, your neurons release a certain number of neurotransmitters. This allows signals to be sent.



When the adenosine receptors are activated, the neuron releases less neurotransmitters. The end result is that your brain stops sending signals to the neurons to tell them to do things, and you feel worn out and tired. **Adenosine acts as an activator of the adenosine receptor, causing less neurotransmitter to be released, and making you feel tired.**



The other is that the concentration of calcium in the cytoplasm gets low. When impulses travel through a neuron, ions move into the neuron. These ions bind to ion receptors on the ER (endoplasmic reticulum) in the neuron. This binding causes the ER to release large amounts of calcium into the cytoplasm. **A high concentration of calcium causes more neurotransmitters to be released.**



If the ion receptor is not activated, or if there are low levels of calcium in the neuron, the vesicles will not release as many neurotransmitters. Ions act as an activator of the ion receptor, and makes your neurons release neurotransmitters.

As described above, adenosine and ions (like Ca^{2+}) can act as neuromodulators. **Neuromodulators** control the amount of neurotransmitters released. The upper motor neuron has to release a certain number of neurotransmitters in order to activate the lower motor neuron. Both adenosine and ions act as activators. They activate their receptors. An inhibitor would be something that binds to the receptor, blocking the receptor, but not activating it.

QUESTIONS

1.3) Define the term, “neuromodulator”

1.4) When the ion receptor is activated, does it result in more or less neurotransmitters being released?

1.5) When the adenosine receptor is activated, does it result in more or less neurotransmitters being released?

PART 2: Coffee before the big race?

Marion, Jesse, Wilma, and Carl, high school track coaches in Winners County, met to discuss the upcoming track meet.

Carl: OK, so it looks like we've got all the details set up, there's just one last issue I'd like to raise and it has to do with some rumors that the students have been spreading around.

Jesse: Oh, like how our team is going to crush everyone?

Wilma: Yeah right. Carl, you're talking about the coffee drinking routine, right?

Carl: Yes, that's right…a number of our team members have been talking about how competitors, at a school I will not name, are going to be visiting Starbucks and ordering espressos right before the meet.

Marion: OK, OK, I know what you're talking about…but what's the big deal? A little coffee beforehand keeps everyone awake and alert. And you know these kids…up all night with excitement, can't sleep. A little jolt will do them good.

Jesse: I get that…but at what point is drinking coffee like doing steroids? Is it a performance enhancing drug? I thought athletics was all about what your body brought to the table, not what extra enhancements can do for you…

Wilma: Well, I don't know…is eating good nutritious food considered performance enhancing? Or vitamins? Or protein supplements?

Carl: Coffee and caffeine aren't steroids, for sure. But if caffeine gives our athletes a chemical edge while it's circulating in their bodies, as opposed to something like protein supplements that help their bodies get strong during training…I would tell the kids they can't do it.

Marion: I suppose…but does it really do that? I mean, caffeine just makes you less sleepy, right? How does it help with a sprint or an endurance run?

QUESTIONS

2.1) What are the coaches concerned about?
2.2) What does Marion want to know?
2.3) What do you need to know to answer Marion's question?

Make some hypotheses…
2.4) Based on your personal experience, do you think caffeine can improve athletic performance?
2.5) Do you think caffeine acts as an inhibitor or activator of the adenosine receptor? Why or why not?
2.6) Do you think caffeine acts as an inhibitor or activator of the ion receptor? Why or why not?

PART 3: Testing Your Hypothesis With The Adenosine Receptor Activation Assay (aka, “a test”)

You will be using a genetically altered cell to test your hypothesis. Cells in this culture have been engineered to produce an adenosine receptor (AR) on their cell membrane that produces a flash of light if the adenosine receptor is activated.

QUESTIONS

3.1) If you add adenosine to the AR cell culture, would you see a flash of light? Explain.

3.2) If caffeine were an inhibitor of the adenosine receptor, would you see a flash of light if you added caffeine to the AR cell culture? Explain.

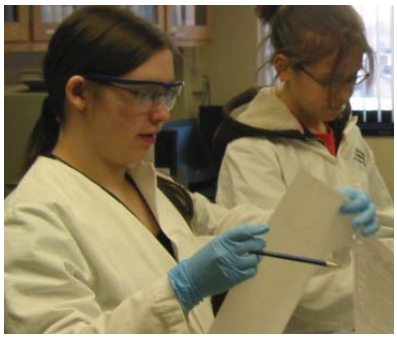
3.3) If caffeine were an activator of the adenosine receptor, would you see a flash of light if you added caffeine to the AR cell culture? Explain.

Laboratory Materials

- 1 plastic cup containing about 10mL “adenosine”
- 1 plastic cup containing about 10mL “caffeine”
- 1 plastic cup containing about 10mL “AR cell culture” squares (cells are applied to squares and kept in cell culture media)
- Forceps
- Droppers
- Plastic tray
- Goggles and gloves

Safety Notes

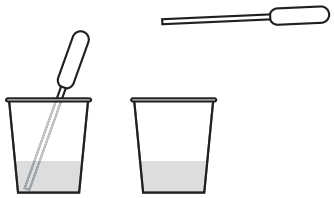
Some of the solutions you will be using can hurt your skin. Wear gloves and goggles and avoid splashing liquid.



Procedure

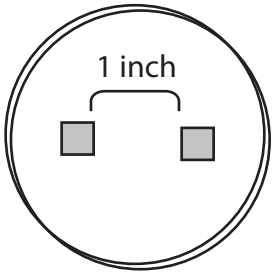
Pay attention, only use one dropper at a time!

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!

1) Each paper square has a layer of AR cells on it. Place two of these squares into your dish, about 1 inch apart.



2) To one of the squares, use a dropper to drop (From a height of about 1 inch) 1 drop of adenosine onto the square.

QUESTIONS

3.4) What happened with you dropped adenosine onto the square of AR cells?

You should have seen a flash of light, because you know that adenosine is an activator of the adenosine receptor. If you did not see a flash of light, see your teacher, as your materials may have been contaminated.

3) To the second square, use a dropper to drop (From the height of about 1 inch) 1 drop of caffeine onto the square.

QUESTIONS

3.5) What happened when you dropped caffeine onto the square of AR cells?

Procedure (Continued)

If you saw light, then you are done, you have identified caffeine as an activator of the adenosine receptor. Proceed to questions 3.7 and 3.8. If you did NOT see light, you still need to figure out if caffeine is an inhibitor…or maybe it doesn't bind to the adenosine receptor at all, so proceed to step 4.

4) To the second square, add ten more drops of caffeine (this will "saturate," or fully fill all the receptors with caffeine).

5) Wait one minute for the receptors to fill.

6) Add one drop of adenosine.

QUESTIONS

(Only answer this if you are completing step 4, if you are not completing step 4, write in your lab notebook, "Not doing this step)

3.6) What happened when you added a drop of adenosine to the AR cells that have caffeine on them?

If you saw light, then caffeine didn't interfere with adenosine's ability to bind to the adenosine receptor. Therefore, caffeine is neither an activator nor an inhibitor. If you did NOT see light, then caffeine is blocking adenosine from binding to the receptor, therefore caffeine is an inhibitor of the adenosine receptor.

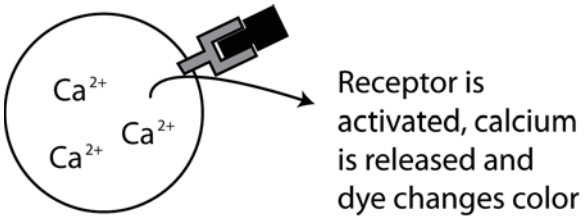
QUESTIONS

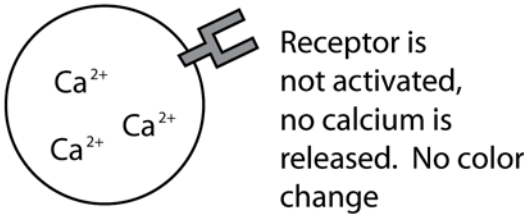
3.7) Is caffeine an activator or inhibitor of the adenosine receptor? Why do you conclude this?

3.8) Could caffeine be acting as a performance enhancing drug through its effect on the adenosine receptor? Why do you think so?

PART 4: Testing Your Hypothesis With The Ion Receptor Activation Assay

Cells in this culture have been engineered to produce an ion receptor (IR) on their cell membrane. In the culture media, there is a dye that detects an increase in calcium concentration. If calcium concentration goes up, as it would if the ion receptor is activated, the color will change. You will be using a molecule called ryanodine to activate the receptor. This molecule is not normally found in the body, but is used by scientists to study ion receptors.





QUESTIONS

4.1) If you add ryanodine to the IR cell culture, would you see a color change? Explain.

4.2) If caffeine were an activator of the ion receptor, would you see a color change if you added caffeine to the IR cell culture? Explain.

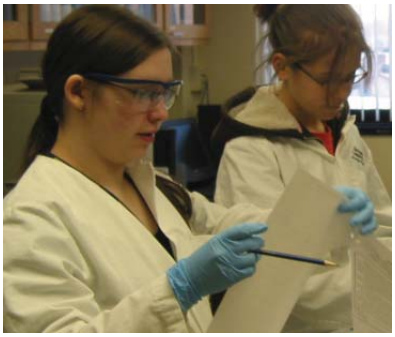
4.3) If caffeine were an inhibitor of the ion receptor, would you see a color change if you added caffeine to the IR cell culture? Explain.

Laboratory Materials

- 1 plastic cup containing about 10mL “ryanodine”
- 1 plastic cup containing about 10mL “caffeine”
- 1 plastic cup containing about 10mL “RR cell culture” squares (cells are applied to squares and kept in cell culture media)
- Forceps
- Droppers
- Plastic tray
- Goggles and gloves

Safety Notes

Some of the solutions you will be using can hurt your skin. Wear gloves and goggles and avoid splashing liquid.



Procedure

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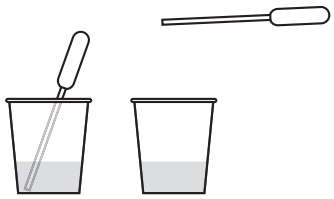
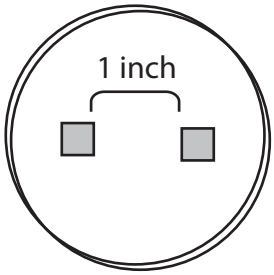


In an effort to conserve, you may be re-using pipettors. As long as one pipettor is always being used for the SAME LIQUID, you should be fine. To avoid putting the wrong dropper in the wrong container, only use ONE dropper at a time. That way, you'll always know which container to put it back in!

1) Each paper square has a layer of IR cells on it. Place two of these squares into your dish, about 1 inch apart.



2) To one of the squares, use a dropper to drop (From a height of about 1 inch) 1 drop of ryanodine onto the square. This is your positive control

QUESTIONS

4.4) What happened with you dropped ryanodine onto the square of IR cells?

You should have seen a color change, because you know that ryanodine is an activator of the ion receptor. If you did not see a color change, see your teacher, as your materials may have been contaminated.

3) To the second square, use a dropper to drop (From the height of about 1 inch) 1 drop of caffeine onto the square.

QUESTIONS

4.5) What happened when you dropped caffeine onto the square of IR cells?

Procedure (Continued)

If you saw a color change, then you are done, you have identified caffeine as an activator of the ion receptor. Proceed to questions 4.7 and 4.8. If you did NOT see a color change, you still need to figure out if caffeine is an inhibitor…or maybe it doesn't bind to the ion receptor at all, so proceed to step 4.

4) To the second square, add ten drops of caffeine (this will "saturate," or fully fill all the receptors with caffeine)

5) Wait one minute for the receptors to fill

6) Add one drop of ryanodine.

QUESTIONS

(Only answer this if you are completing step 4, if you are not completing step 4, write in your lab notebook, "Not doing this step)

4.6) What happened when you added a drop of ryanodine to the IR cells that have caffeine on them?

If you saw a color change, then caffeine didn't interfere with ryanodine's ability to bind to the ion receptor. Therefore, caffeine is neither an activator nor an inhibitor. If you did NOT see a color change, then caffeine is blocking ryanodine from binding to the receptor, therefore caffeine is an inhibitor of the ion receptor.

QUESTIONS

4.7) Is caffeine an activator or inhibitor of the ion receptor? Why do you conclude this?

4.8) Could caffeine be acting as a performance enhancing drug through its effect on the ion receptor? What makes you think so?

PART 5: What's The Evidence?

Carl: OK…so there is evidence that there is a biological mechanism for how caffeine can actually enhance muscle activity. It can definitely affect receptors in our body that regulate how tired we feel and how messages get sent from our brain to our muscles. That qualifies as a performance enhancing drug to me.

Wilma: I say we tell kids they can't drink coffee or caffeinated drinks before a competition.

Marion: Now wait a minute, just because it's BIOLOGICALLY possible doesn't mean that it actually works right? I mean, it's BIOLOGICALLY possible that we can genetically engineer away a lot of genetic diseases, but you don't see that working in people right now. Chemotherapy is based on a really good biological mechanism…but it doesn't work all the time either.

Jesse: Marion is right…maybe we should have a look at what the research says. Here is an article that summarizes a lot of studies where people took caffeine and tried to do different athletic activities. This article has been "peer-reviewed." That means that other scientists have read it before it was published and decided that the study was done well and their conclusions make sense.

Wilma: OK, let's take a look....

Sports Med 2001; 31 (11): 785-807
0112-1642/01/0011-0785/$22.00/0

Caffeine and Exercise
Metabolism, Endurance and Performance

Terry E. Graham

Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada

Table III. A summary of studies that examined the effects of caffeine on performance

Reference	Participants	Caffeine dose (mg/kg)	Protocol	Key results
Ivy et al.[42]	7 M; 2 F; trained cyclists	250mg + 250mg (M 6.9, F 8.8)	Cycle for 2h to produce greatest amount of work possible	Caf resulted in 7.4% more work done; 31% more fat oxidised; glu polymer ingestion had no effect on work done
Cohen et al.[43]	5 M; 2 F; trained runners	(a) 0; (b) 5; (c) 9	Run 21km in hot, humid environment	No differences in run times
Berglund & Hemmingsson[44]	8-10 M; 4-5 F; trained skiers	6	n = 13 raced 23km at altitude; n = 14 raced 20km at sea level. Both were 2 lap courses	Race time ≈55 and 67 min for M and F. All 1 and 2 lap times were faster* with caf except for 2 laps at low altitude ($p < 0.10$)
Kovacs et al.[31]	15 M; trained cyclists	(a) 0; (b) 0; (c) 2.1; (d) 3.2; (e) 4.5	Complete a simulated time trial estimated to last about 1h	Time (min): (a) 62.5; (b) 61.5; (c) 60.4;* (d) 58.9;** (e) 58.9**
MacIntosh & Wright[45]	7 M; 4 F; trained swimmers	6	Swim 1500m	Split times caf faster by: 500m ≈7 sec;* 1000m ≈8 sec;* 1500m 23 sec;* (20 : 58.8 *vs* 21 : 21.8 min)
Bruce et al.[46]	8 M; trained rowers	(a) 0; (b) 6; (c) 9	Simulated rowing 2000m	Time (sec): (a) 416; (b) 411;* (c) 412*
Wemple et al.[30]	4 M; 2 F; active individuals	Glu + ele ± 8.7 caf	60% of max for 3h followed by 500 rpm at high resistance	Time (sec) for 500 rpm: pl 343; caf 344
Wiles et al.[23]	18 M; 10 M; trained runners	Decaf or reg cof (≈2-2.5)	Simulated 1500m run; (a) run 1500m while controlling speed; (b) run 1100m at 'controlled' speed and then 'kick' to finish	(a) total time (sec); pl 290.2; coffee 286.0;* (b) final 400m (km/h); pl 22.9; coffee 23.5*
Collomp et al.[47]	Trained: 3 M; 4 F; untrained: 2 M; 5 F	250mg (≈4.3)	Swim 2 × 100m freestyle with 20 min recovery	Trained: caf resulted in ≈1 sec improvement* in both swims. Untrained: no change in speed
Collomp et al.[48]	3 M; 3 F; 'active'	5	One Wingate test, i.e., 30 sec 'all-out' cycling	No difference in peak, average power or in rate of fatigue
Greer et al.[49]	9 M; 'active'	6	4 Wingate tests with 4 min rest	No differences in peak, average power or in rate of fatigue
Anselme et al.[50]	10 M; 4 F; 'active'	250mg (≈3.6)	Repeated 6 sec cycle sprints (5 min rest) with progressively greater resistance	Caf: max power 964 *vs* 904W*

caf = pure caffeine; **decaf** = decaffeinated coffee; **ele** = electrolytes; **F** = female; **glu** = glucose; **M** = male; **pl** = placebo; **reg cof** = regular coffee; * indicates that the difference was significant; ** indicates that the results from this treatment were significantly different from those without **.

Carl: WHAT?!!! Are you kidding me? I can't figure that out!! What kind of idiot makes a table like that? I can't make heads or tails of it! I don't have time to get a degree in science to figure that out.

Florence: Hang on there Carl...breathe in and out. Let's rewrite this a little to simplify it. First off...I want to take out the studies that only look at men. You don't see all men around this table, and you certainly don't see only men on our track teams! (crosses off the studies with only M)

Jesse: OK, that leaves us with eight studies now

Marion: I think I see where Florence is going with this -- let's just make a table that makes sense to us...we'll identify each study, or reference, by the first name listed, just to make it simple. Then we'll make a column for participants, but since all of them have men and women, we'll use it to identify whether or not someone is trained in an event, or if they're just active

Jesse: And then, we can probably leave out the dose of caffeine. It's not like we're comparing caffeine to adrenaline or anything else. Just different doses of caffeine.

Carl: OK, I see...and the doses of caffeine are all between about 2-9mg/kg. So on average, say, 5mg/kg. I weigh 150 pounds, so that's 68kg. That means, I would have gotten a dose of 340mg of caffeine. That's like drinking four cans of Red Bull.

Florence: And let's simplify the "protocol" column to just what the activity was and how far or how long they did it.

Marion: And we will rewrite the "key result" column to "was it significant to have caffeine?" and add a column telling us what they measured...like time of a run, or amount of work.

Carl: How do we know if it's significant?

Jesse: We look at the bottom of the table for the footnotes...if a study has an asterisk, one or two asterisks, it means it's significant.

Florence: Allright, let's make a new table. Much better than Dr. Graham's I think!

Carl: Maybe not better, but at least I can look at it without wanting to throw up.

QUESTIONS

5.T) Draw this table in your lab notebook. Fill in the blank spots using the information in the original article.

Reference (author of study)	Are participants specialists in any sports?	What did the participants do?	What was measured in the key results?	Was the result significant?
Ivy	Trained cyclists	Cycle for 2 hours	Work and amount of fat oxidized	No
Cohen	Trained runners	Run 21km (hot and humid)	Time	No
Berglund				
MacIntosh				
Wemple				
Collomp				
Collomp				
Anselme				

Marion: So how many significant studies are there?

Jesse: Let's just highlight all the studies that used trained athletes and just measured time. Afterall...our students are trained, and their success is measured in time. I see something odd here...

Carl: And what about that race was in a "hot and humid" environment. Does that make it unusually difficult, so it's like comparing apples to oranges?

Marion: So...if you're a trained athlete versus just an active person, does caffeine do things differently? And if you're trying to lift weights and produce power, or trying to lose weight, maybe it does and maybe it doesn't help? What do you all think?

QUESTIONS

5.1) What is the main purpose of the table?

5.2) How many studies looked at both male and female athletes?

5.3) Based on the information in the table, what conclusions can be drawn about how caffeine affects the performance of trained athletes?

5.4) Based on the information in the table, what conclusions can be drawn about how caffeine affects the performance of untrained, but active individuals?

5.5) Caffeine is no longer banned by the world anti doping agency. Do you think it should be banned? Why or why not? Write a **one paragraph** explanation.

5.6) What do you think about this statement from Terry Graham (shown below)? Write a **one paragraph** response.

"Athletes who ingest caffeine are using a drug for the express purpose of gaining an advantage. As such, the author considers it to be doping and unethical. If an athlete has made a conscious decision to take caffeine for the purpose of gaining an advantage and enhancing performance, this could be the first of a series of similar decisions for other drugs."

CAREERS AND SCIENCE

OLYMPIC HOPEFUL

Titles may mean the world to an athlete, but Sam Gunatileka takes away more than that from the badminton court. The 28-year-old test engineer and badminton player has had an enviable amount of international fame: he was the US Junior National Champion in 2003; he and his partner, Vincent Nguy, won the Pan American Badminton Championships in 2010; and he has represented the US in many tournaments, putting him in contention as one of the players to represent Team USA at Olympics 2012. Having played badminton since he was 10, Sam sees badminton as a way to clear his mind of stress, as well as a social activity that brings people closer on the court, since badminton is played worldwide and open to people of all ages and levels of ability.

Sam's passion for the game is admirable, as he has to juggle a 9-to-5 job and constant training, not to mention classes and homework during his college years. Training for the Olympics took three and a half months off his work schedule, when he traveled to Thailand and Malaysia to train under renowned coaches and practice with world-class players. This was on top of his year-round travels to tournaments, and some could take one-and-a-half weeks. It is hard to imagine any boss being okay with this work schedule, but Sam's ability to get his work done on time earned him those days off. Though he now lives in San Jose, CA, Sam previously lived and trained in Maryland, where he and his partner did not have the luxuries that players from the West Coast enjoyed, from sponsors and training facilities to high ranking coaches. As such, beating West Coast players in competitions has always been a point of pride for Sam, and it victories like these that spurred him on.

Hand-eye coordination matters greatly in a sport that requires quick reflexes and for players to keep their eyes on the ball. Sam develops his abilities through repetition, hitting a shuttle (aka "birdie") 200 times into different spots onto the court over a long period of time. He finds that his motor coordination improves the more games he plays. What does not help his control of motor skills is, in fact, caffeine. Even though caffeine was removed from the list of prohibited substances by World Anti-Doping Agency (WADA) in 2004, Sam chooses to stay away from caffeine because he finds that it affects his performance negatively on the court by reducing his stamina, slowing his reaction time and giving him gas problems! He prefers sports drinks which replenish the body's water and electrolyte levels. His personal observations are supported by research. Caffeine has been shown to improve athletic performance most consistently for athletes engaging in high-intensity exercise over long periods of time. A typical point in badminton lasts only several seconds, but in those few seconds, a shuttle trades sides around 10 times (compared to 3-4 in tennis) and can travel around up to 200mph. Badminton is more like sprinting than a marathon, and caffeine has not been shown to be as effective in improving sprint times or performance. So it looks like Sam is just going to have to rely on his training and guts to go for the gold!

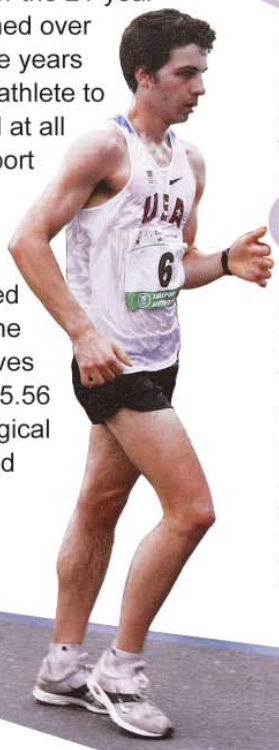
Hand-eye coordination is the ability to coordinate the information received through your eyes to control, guide and direct the hands to execute a specific task.

Aside from the money he wins at tournaments, Sam isn't paid to play badminton. If he plays a tournament sponsored by the United States Badminton Association, he could win $1,000 for taking first place in doubles, or $1,400 for a first place finish in singles.

Careers and Science
Racewalker

Racewalking is a sport that reminds us of the skills required of hunter-gatherers 10,000 years ago, when humans often had to chase their prey to the point of exhaustion, covering over 25km (14.9 miles) in two to five hours. The fastest time recorded for a 20km (12.4 miles) racewalk is 1 hour, 16 minutes and 43 seconds – a record held by Sergey Morozov from Russia. This athletic event requires racewalkers to achieve the stride rate of an Olympic 400-meter runner, but for hours at a time. Racewalker Dan Serianni's fastest recorded time for the 20km walk is 1 hour and 30 minutes, which has qualified him for six international track and field events. Since then, he has competed within the country, in Canada, Colombia, Russia and, soon, Mexico. His most memorable experience was competing in Saransk, a small Soviet-era industry town outside of Moscow, where he gave out t-shirts to children who had specially requested for "American t-shirts".

These are impressive achievements for the 21-year-old from Rochester, NY, who only switched over from running events to racewalking three years ago. Because racewalking requires the athlete to have one foot in contact with the ground at all times, racewalking is a very technical sport that requires good gross motor skills. Knowing how to position your arms when walking can give you bigger or more strides per minute. Dan has learned to condition his body to walk as fast as he can over a long distance. Part this involves his self-designed 10-hour and 120km (75.56 miles) training per week. A neat neurological trick that Dan discovered is the uphill and downhill repeats. Uphill prepares you mentally for the flat race track, which is easier to walk on; walking downhill allows for faster strides, which gets the neurons and muscles conditioned to firing faster.



So what about chemical conditioning? The year after setting his world record, Sergei Morozov was tested positive for the EPO, a performance-enhancing drug that improves oxygen delivery to muscles. He was banned from competitions for a year and his world record was never formally confirmed. Does using caffeine give track and field athletes an unfair advantage over those who do not? Yes and no. The National Collegiate Athletic Association (NCAA) limits caffeine consumption to 500mg, because it can enhance performance by increasing availability of stored fat and conserving glucose. According to Dan, however, caffeine is not that important to sprinters and even the 20km race-walkers. A study has shown there isn't a big enough increase in muscular output when athletes take caffeine, and Dan thinks it gives you more of a mental boost instead of having significant effects on muscles. Caffeine is also relatively cheap; many athletes can purchase it, which levels the playing field. Drugs like EPO are usually banned because they are expensive, dangerous and highly effective.

Where caffeine does make all the difference is in the 50km (31.07 miles) race walk. Before sports drinks like Gatorade were made available, pit stops stocked up on defizzed Coca-Cola. Within the 25km to 35km mark, athletes experience extreme neural and muscle fatigue, having used up all the simple sugars in the body. To these athletes, caffeine – with its ability to activate ion receptors in the neuron so as to fire up the muscles again – makes the difference between getting to the finish line and not. Performance-enhancing? Maybe.

Aiding with recovery so the athlete does not keel over mid-race? Definitely.

Many Olympics title holders start at a young age and train all year round for extensive hours. Having a coach matters less in this sport, as it is about getting your body used to walking long distances, and the adage "miles make champions" holds true for racewalking. Getting involved in local races and marathons will help you get better and be selected for higher-level competitions.

EPO stands for Erythropoietin, which controls red blood cell production.In sports, blood doping is when drugs like EPO are used to increase the number of red blood cells, which improves oxygen delivery to muscles and directly increases an athlete's aerobic capacity and endurance.