Supplemental Material

CBE—Life Sciences Education

Dasgupta *et al.*

Supplement A: Modified Glossary of Terms

(In alphabetical order and posted online at http://www.scicard.net/glossary/index.php)

Note: Underlinesindicatemodificationsinformed by 'Neuron Assessment' findings to glossary items fromDasgupta et al., *CBE Life Sci Educ* 13: 265-284, 2014.

1. **Control:** An experimental baseline against which an effect of the treatment conditions may be compared (Holmes, Moody & Dine, 2011). The control is represented on the x-axis next to the treatment for comparison in a bar graph or as a comparison set of data in the graph.

2. **Control group:** A control group of experimental subjects or units, for comparison purposes, measures natural behavior under a normal condition instead of exposing them to experimental treatment conditions. Parameters other than the treatment variables are identical for both the treatment and control conditions. (Gill and Walsh, 2010; Holmes, Moody and Dine, 2011).

3. **Correlation relationship:** Two variables are said to be correlated if an observed change in the level of one variable is accompanied by a change in the level of another variable. The change may be in the same direction (positive correlation) or in the opposite direction (negative correlation). Note that correlation does not imply causality. It is possible for two variables to be associated with each other without one of them causing the observed behavior in the other. When this is the case it is usually because there is a third (possibly unknown) causal factor (NIST/SEMATECH, 2003)

4. **Cause and effect relationship:** There is a causal and effect relationship between two variables if a change in the level of one variable (independent variable) causes an effect in the other variable (dependent variable). To establish a cause and effect relationship, one must gather the data by experimental means, controlling unrelated variables which might confound the results. Having gathered the data in this fashion, if one can establish that the experimentally manipulated variable is correlated with the dependent variable, then one should be (somewhat) comfortable in making a causal inference. That is, when the data have been gathered by experimental means and confounds have been eliminated, correlation does imply causation (NIST/SEMATECH, 2003; Wuensch, 2001). The causal relationship would be coherently interpreted from a graphical representation if one is included.

5. **Factors:** the specific treatments or experimental conditions (the independent variables) (Dasgupta et al., 2014). These are identified in a key, the symbols, and the figure legend in a report of experimental research findings.

6. **Hypothesis**: A testable statement that carries a predicted association between a treatment and outcome variable. An investigator designs an experiment to test the hypothesis, and

the experimental results are used to evaluate the hypothesis for confirmation or refutation (Ruxton & Colegrave, 2006).

7. **Outcome (dependent) variable:** A factor under investigation where it is reasonable to argue that there may be a relationship with an independent variable. The dependent variable is measurable in terms of units. (Holmes, Moody & Dine, 2011). In a graph, values for outcome variables would be on the y axis.

8. **Outside/unrelated/control/confounding variables:** Any factors (s) that may influence your observations/experiment but is not the factor you are investigating. (Holmes, Moody & Dine, 2011).

9. **Population:** All individuals of a defined group appropriate for collecting information for a particular investigation goal (Dasgupta et al., 2014).

10. **Random (representative) sample:** A sample where all experimental subjects from a target demographic have an equal chance of being selected in the control or treatment group.

11. **Randomization:** A random sample is selected from a target population; units are then assigned to different treatment groups (Ramsey & Schafer, 2002).

12. **Replication:** Replication is performed to assess natural variability, by repeating the same manipulations to several experimental subjects (or units carrying multiple subjects), as appropriate under the same treatment conditions (Quinn & Keough, 2002).

13. **Sample:** A random (smaller) group of representative individuals selected from the population, from which data is collected and conclusions are drawn about the population (Dasgupta et al., 2014).

14. **Sample size:** An appropriate representative sample size is one that averages out any variations not controlled for in the experimental design (The College Board, 2006).

15. **Scope of inference**: Recognizing the extent and limit of inferences that can be made from a small characteristic sample of experimental subjects or units to a wider target population and knowing to what extent findings at the experimental subject level can be generalized.

16. **Subject:** The individuals to whom the specific variable treatment or experimental condition is applied. Each experimental subject carries a variable property (Dasgupta et al., 2014). Subjects are identified in the title or figure caption of a graph.

17. **Treatment (independent) variable:** The factor(s) in your experiment whose effect you are examining (Holmes, Moody & Dine, 2011). Treatment variables are presented as column in a table and alongside control group variables on a graph.

18. **Treatment group:** A group of experimental subjects or units that are exposed to experimental conditions varying in a specific way (Dasgupta et al., 2014).

19. **Unit:** The group of individuals to which the specific variable treatment or experimental condition is applied (Dasgupta et al., 2014)

20. **Variable:** A certain property of an experimental subject that can be measured and that has more than one condition (Dasgupta et al., 2014).

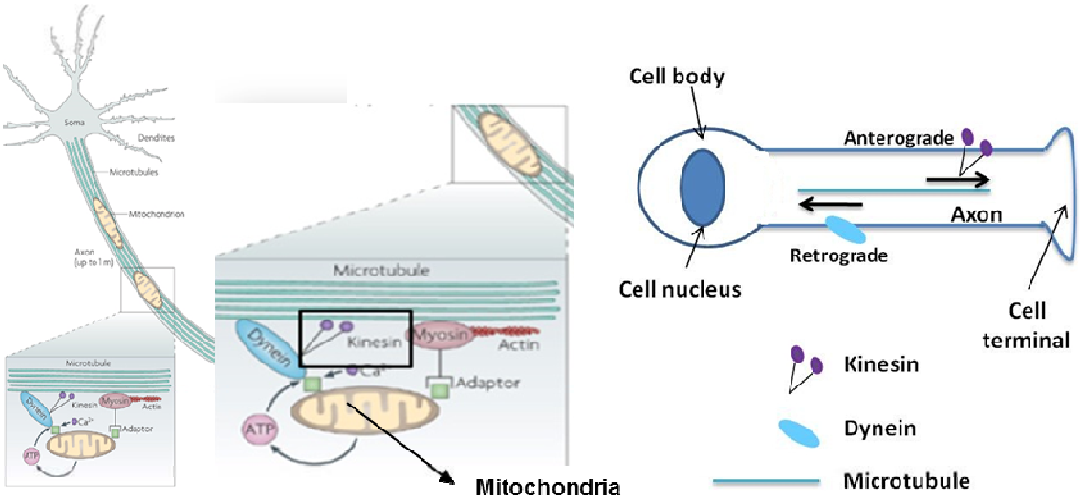
21. **Variation:** when observations within your data set do not all have the same value (Holmes, Moody & Dine, 2011). Variations in data can be accounted for by using measures from strategies like randomization and replication.

22. **Variability:** sources of variability in the experimental design of biological study are often divided into two categories: biological variability (variability due to subjects, organisms, and biological samples) and technical variability (variability due measurement, instrumentation, and sample preparation) (Box et al. 2005; Cox and Reid 2000). On a graph representing averages of experimental outcome findings, errors bars would represent variability of results from replication of treatments.

Supplement B: 'Neuron Assessment' Answer

Note: This is a typical answer but not the only way to get a correct answer

Figures



(a) (b) (c)

Background

Mitochondria are one of the several organelles that get transported across the axon of a nerve *(Refer figure above)*. They are transported in both directions along the length of the axon. The movement of mitochondria from the cell body to the cell terminal is termed as anterograde transport while the movement from the cell terminal to the cell body, in the opposite direction, is termed as retrograde transport. Movement of mitochondria takes place on the microtubules present along the length of the axons. This complex movement is facilitated by the interaction of motor proteins, kinesin and dynein, present in the axons.

Directions

Medical researchers at Seattle Grace Hospital are trying to diagnose the cause for a disorder caused by impaired mitochondrial movement within neurons in human subjects. Cell culture studies have been performed to observe the movement of mitochondria within neurons.

The researchers think that kinesin or dynein activity might play a role in the cause of this disorder. Pretend that you work for a company called *MedResearch* that has been assigned to design an experiment to test how kinesin or dynein can affect mitochondrial movement. In your lab you have the following chemicals:

Compound K: inhibits kinesin;

Compound D: inhibits dynein;
Image software: measures mitochondrial movement in neurons.

- **How do you think a 'hypothesis' relates to an experiment?**

A hypothesis is testable outcome of an experiment and defines the relationship between independent (treatment) and dependent (outcome) variables within an experiment.

1. **Describe what you see in the three diagrams above. Please tell us in detail what you think about it.**

In the left most Figure, I see the figure of an axon and mitochondria present within it. The figure in the middle is a magnified version of the mitochondria attached to microtubules via several motor proteins. The figure on the extreme right shows kinesin and dynein motor proteins that are involved in movement in the anterograde and retrograde direction respectively. The three figures together show the mechanism of movement of mitochondria along an axon with the help of motor proteins like kinesin and dynein.

2. **What could be a potential hypothesis for your experiment?**

Inhibition of kinesin and/or dynein will stop movement of mitochondria along the axon.

3. **Which factors will you vary and which will you keep the same in your study? Why?**

I would start off varying kinesin activity using compound K and observe its effect on mitochondrial movement in the anterograde direction towards the cell/axon terminal. Next I would wash off compound K to restore kinesin activity and vary dynein activity by using compound D to inhibit it. Then, I would measure movement of mitochondria in the retrograde direction. I can also use compound K and D together to see if movement of mitochondria is completely stopped across the neuron. The neuron source and other variables like calcium concentration, ATP molecules should be maintained as close as possible to reduce the effect of any confounding variables.

4. **How will you assign subjects to groups for your experimental study? Explain.**

I will ensure that I select neuronal cell cultures from pool of subjects that are representative of a larger population that the study will be applicable to. I will assign cell cultures to an **experimental** and a **control** group in my study. Cultures will be assigned to either of the groups using random sampling. The control groups cell cultures will not be treated neither compound K nor D. The experimental group will consist of cell cultures that will be treated with compound K and/or compound D.

5. **Do you think you can establish a cause-and-effect relationship between the treatment and a response variable in this experiment? Justify your answer.**

Yes I think a cause and effect relationship can be established between inhibition of kinesin or dynein using compound K or D (treatment) and effect of movement of mitochondria (response) if: Inhibition of kinesin using compound K stops anterograde movement; inhibition of compound D using dynein stops retrograde movement; using compound K and D in combination will complete stop or allow minimal mitochondrial movement across neurons.

6. **How would you present the results of your experiment?**

I would present the results with the help of a graph that will include mean mitochondrial movements towards the cell terminal (after using Compound K to inhibit Kinesin) and towards the cell body (after using Compound D to inhibit Dynein). I will also have errors bars for bars on my graph to represent mitochondrial movement variations as a result of replication of treatments.

7. **What results do you expect to get and what would those mean? Using complete sentences, explain what criteria will be used to indicate the success or failure of your experiment.**

I would expect to see inhibition of kinesin result in a slowing of anterograde movement while inhibition of dynein would result in a slowing of retrograde movement. I also expect the combination of the two inhibitors would prevent any mitochondrial movement. These expectations would be validated through the use of microscopy and a digital measurement of the distance traveled.

8. **How will you improve the validity of your experiment?**

The findings of this experiment can be improved by repeating /replicating treatments. Also, conducting the experimental study on sample of subjects that are representative of a larger population of human subjects increases the experiment reliability.

9. **What do you think this diagram is not showing? Explain your answer.**

The diagram fails to show how the motor appears during each of the two directions of motion. But together with the figures and the background, the question has all the details necessary to answer the questions given.

10. **Is there anything about this question that you don't understand or find confusing? Explain.**

Not necessarily. I know you did it to simplifying the context but I believe a large body of initial work would be required to get to narrowing down to kinesin or dynein being responsible for the disorder. So in a way I like that the question makes it easy by ruling out any other possibilities because just by itself, mitochondrial transport impairment could be potentially due to a host of things.

11. **Consider yourself a diagram designer. If you could change the diagrams, what would you change or how would you improve them?**

The figures by themselves are OK. I know it doesn't include any measurement values because part of the question was for the students to think about that aspect. If you were to think about a classroom activity using this question, you would have the students go through the background information and perhaps sketch out plots and have that as supplement to the text.

Supplement C Table 1: Rubric for Experimental Design (RED) Modified with Graphical Representation Abilities

Note: Underlinesindicatemodifications to RED from Dasgupta et al., *CBE Life Sci Educ* 13: 265-284, 2014.

Areas of Difficulty	Propositional Statements/Completely Correct Ideas	Typical Evidence of Difficulties
(1) Variable property of an experimental subject	**Experimental subject or units:** The individuals to which the specific variable treatment or experimental condition is applied. An experimental subject has a variable property. A variable is a certain property of an experimental subject that can be measured and that has more than one condition.	a. An experimental subject was considered to be a variable. b. Groups of experimental subject were considered based on a property *that diverges* from the subjects that were the target for the stated investigation or claim to be tested. c. Variable property of experimental subject considered is not consistent throughout a proposed experiment.
	Graphical representation: Experimental units or subjects are identified in a title or the figure caption.	d. The experimental subject was represented as a treatment group along the x-axis.
(2) Manipulation of Variables	**Testable hypothesis:** A hypothesis is a testable statement that carries a predicted association between a treatment and outcome variable.	a. Only the treatment and/or outcome variable is present in the hypothesis statement. b. Hypothesis does not clearly indicate the expected outcome to be measured from a proposed experiment.
	Treatment group: A treatment group of experimental subjects or units is exposed to experimental conditions that vary in a specific way.	c. Haphazard assignment of treatments to experimental units in a manner inappropriate for the goal of an experiment. d. Treatment conditions proposed are unsuitable physiologically for the experimental subject or inappropriate according to the goal of an investigation.
	Combinatorial reasoning: In experimental scenarios when two or more treatment (independent) variables are present simultaneously, all combined manipulations of both together are examined to observe combinatorial effects on an outcome.	a. Independent variables are haphazardly applied, in scenarios when the combined effects of two independent variables are to be tested simultaneously. b. Combining treatments in scenarios where the effect of two different treatments are to be determined individually.

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Areas of Difficulty	Propositional Statements/Completely Correct Ideas	Typical Evidence of Difficulties
	Controlling outside variables: The control and treatment groups are required to be matched as closely as possible to equally reduce the effect of lurking variables on both groups.	c. Variables unrelated to the research question (often showing a prior knowledge bias) are mismatched across treatment and control groups.
	Control group: A control group of experimental subjects or units, for comparison purposes, measures natural behavior under a normal condition instead of exposing them to experimental treatment conditions. Parameters other than the treatment variables are identical for both the treatment and control conditions.	d. The control group does not provide natural behavior conditions because absence of the variable being manipulated in the treatment group, results in conditions unsuitable for the experimental subject. e. Control group treatment conditions are inappropriate for the stated hypothesis or experiment goal.
		f. Experimental subjects carrying obvious differences are assigned to treatment vs. control group.
	Graphical representation: Both treatment and control group are presented as a column in a table and represented side by side on the x-axis in comparison to the treatment group in a graph or as a comparison set of data in the graph	g. Appropriate control and/or treatment groups are not presented alongside treatment groups in tables or graphs.
(3) Measurement of experimental outcome	Treatment and outcome variables should match up with proposed measurements or outcome can be categorical and/or quantitative variables treatments. -A categorical variable sorts values into distinct categories. -A quantitative or continuous variable answers a "how many?" type question and usually would yield quantitative responses.	a. No coherent relationship between a treatment and outcome variable is mentioned. b. The treatment and outcome variables are reversed.

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Areas of Difficulty	Propositional Statements/Completely Correct Ideas	Typical Evidence of Difficulties
	Outcome group: The experimental subject carries a specific outcome (dependent variable) that can be observed/measured in response to the experimental conditions applied as part of the treatment.	c. Outcome variables proposed are irrelevant for the proposed experimental context provided or with the hypothesis. d. Stated outcome not measurable. e. No measure was proposed for the outcome variable. f. An outcome variable was not listed for an investigation.
		g. There is a mismatch between what the investigation claims to test and the outcome variable.
	Graphical representation: In a graph, appropriate outcome variables would be on the y axis.	h. The outcome variable is not represented on the y-axis. i. No units are represented for variable represented on the y-axis
(4) Accounting for variability	Experimental design needs to account for the variability occurring in the natural biological world. Reducing variability is essential to reduce effect of non-relevant factors in order to carefully observe effects of relevant ones.	a.Claims that a sample of experimental subjects will eliminate natural variability with those subjects.
	Selection of a random (representative) sample: A representative sample is one where all experimental subjects from a target demographic have an equal chance of being selected in the control or treatment group. An appropriate representative sample size is one that averages out any variations not controlled for in the experimental design. (NYSED, 2006)	b. Criteria for *selecting* experimental subjects for treatment vs. control group are biased and not uniform. c. Criteria for selecting experimental subjects for investigation are different in a way that is not representative of the target population.

Supplement C Table 1: Rubric for Experimental Design (RED) Modified with Graphical Representation Abilities

Note: Underlinesindicatemodifications to RED from Dasgupta et al., *CBE Life Sci Educ* 13: 265-284, 2014.

Areas of Difficulty	Propositional Statements/Completely Correct Ideas	Typical Evidence of Difficulties
	Randomized design of an experiment: Randomizing the order in which experimental subjects or units experience treatment conditions as a way to reduce the chance of bias in the experiment. Randomization can be complete or restricted. One can restrict randomization by using block design which accounts for known variability in the experiment that can't be controlled.	Decisions to *assign* experimental subjects to treatment vs. control group are not random but biased for each group. d. Random assignment of treatments is not considered. e. Random assignment of treatments is incomplete as they show random assignment of the experimental subjects but instead, what is needed is random assignment of treatments.
	Replication of treatments to experimental units or subjects: Replication is performed to assess natural variability, by repeating the same manipulations to several experimental subjects (or units carrying multiple subjects), as appropriate under the same treatment conditions.	f. Replication means repeating the entire experiment *at some other time* with another group of experimental subjects. g. No evidence of replication or suggested need to replicate as a method to access variability or to increase validity/power of an investigation.
	Graphical Representation: On a graph representing averages of experimental outcome findings, errors bars would represent variability of results from replication of treatments.	h. Missing error bars on graphs representing averages of experimental outcome findings on y-axis.
(5) Scope of inference of findings	**Scope of inference:** Recognizing the limit of inferences that can be made from a small characteristic sample of experimental subjects or units, to a wider target population and knowing to what extent findings at the experimental subject level can be generalized.	a. The inference from a sample is to a different target population. Usually students overestimate their findings beyond the scope of the target population. b. No steps are carried out to randomly select experimental subjects' representative of the target population about which claims are made.

Supplement C Table 1: Rubric for Experimental Design (RED) Modified with Graphical Representation Abilities

Note: Underlinesindicatemodifications to RED from Dasgupta et al., *CBE Life Sci Educ* 13: 265-284, 2014.

Areas of Difficulty	Propositional Statements/Completely Correct Ideas	Typical Evidence of Difficulties
	Cause and effect conclusions: A cause-and-effect relationship can be established as separate from a mere association between variables only when the effect of lurking variables are reduced by random assignment of treatments and matching treatment and control group conditions as closely as possible. Appropriate control groups also in comparison to the treatment group also need to be considered.	c. A causal relationship is claimed even though the data shows only association between variables. Correlation does not establish causation.
	Graphical Representation: The causal relationship would be coherently interpreted from a graphical representation if one is included.	d. A causal relationship (separate from a mere association) could not be gleaned statistically from the graph because appropriate control groups were not represented on the x-axis in comparison to the treatment group in a graph. e. A causal relationship could not be derived as the patterns between the treatment and outcome group were represented as different from the provided experiment background.

Supplement D: Interview Questions modified from the Schönborn & Anderson (2009) Three Phase Seated Interview Technique (3P-SIT)

Phase 1: *Investigation of prior<u>knowledge</u> about the context (neurons and organelle movement) and experimental design <u>before</u> being exposed to the diagrams and background information.*

1.1. What comes to mind when I say 'neurons'?

1.2. What comes to mind when I say 'organelle movement along neurons'?

1.3. Please draw to help me understand what you mean.

1.4. Would mitochondria perhaps be in the picture somewhere?

1.5. How do scientists know the ideas that you are telling me?

1.6. What would an experiment have involved? What would they have used?

1.7. Would they have measured something? Please explain so I know more about what you are thinking.

Phase 2*: Use of experimental design knowledge to design an experiment in the context of the diagrams and background information presented by the 'Neuron Assessment.'*

2.1. What are your thoughts about what is represented in this figure?

2.2. Why do you think this shows organelle or mitochondrial movement in a neuron?

2.3. What are the scientist/researchers trying to do in this study?

2.4. What would an experiment have involved? What would they have used?

2.5 What would the scientists have measured?

2.6. How will you use materials to conduct your experiment step by step?

2.7 What kinds of treatments will you assign?

2.8 How would you decide on the right sample to be included in your treatment/control group in your study?

2.9. What results do you expect to get and how would you record those?

2.10. Can you please share how you would represent this experiment in a graph? List the values and units of measure in your graph.

2.11 Please explain what you draw as your graph here.

2.12 Earlier you mentioned about some treatment groups. Which of those are you representing in your graph?

Phase 3: *Evaluate and critique the 'Neuron Assessment' and the activity, thereby allowing the researchers to understand their knowledge and validate any difficulties with prior knowledge and experimentation exposed in the first 2 phases.*

3.1. How would you rate the questions about experiments on a 1-10 scale and why?

3.2. Is there anything about the experiment in particular that you don't understand or find confusing?

3.3. What do you think is left out of these questions about experiments? Explain your answer.

3.4. Consider yourself a question designer or textbook author. If you could change this question in any form, what would you do to improve it, if anything?

3.5. Do you think this is a good and clear question? Give reasons for your answer.

3.6. Comment on these types of questions in general, and your feelings on interpreting them.

Supplement E: Interview transcripts

1. Interview Transcript for Expert [Eric]

Interviewer: AD; Eric: E

Phase 1

AD: Hi! Eric, I am Annwesa Dasgupta (AD). How are you doing today?

E: Good!

AD: So, thank you for being here today. Alright, I would like to briefly explain some details about this activity. You just spent some time writing ideas about what you think about experiments. Now I would like to follow up your ideas by giving you an opportunity to share some thoughts verbally via a conversation based on a few questions. Are you ready to begin?

E: Yes!

AD: So, most of my questions will be related to the written survey you just completed, to help me understand your ideas. Some instructions to get started…please think freely about the questions I ask …there are no time limitations so you are free to take as much time as you wish to respond. There is no right or wrong answer to these questions. I am simply interested in your thinking about experiments. You are free to use provided materials to draw things if that helps you to express your thoughts. However, there might be certain instances where I request to visually present your ideas just so I am sure that I understand correctly. This interview will be recorded. You may choose to withdraw your participation at any time without penalty. If you have questions or need clarification at any time during this conversation, please let me know.

AD: let's talk a little bit about neurons. What's the first thought that cross your mind when I mention "neuron"?

E: when you say "neuron", I can picture a few different morphologies of the cell and the synaptic connections between them, the neuron networks with neurons is the basis of that.

AD: So how would you visually represent these ideas?

E: Let's see, I would probably draw... *(Starts drawing Figure 3A)*

I would draw dendrites, an axon and I will make the axon myelinated. I am drawing a circular soma and some dendritic branches going up. I would make couple of terminals, terminal boutons and the en passant bouton. I will leave off the post synaptic boutons for the moment. Then there would be dendrites which I would see in the inferior colliculus inside the auditory thalamus. Often in textbook, the spinal motor neurons are shown as the representative neurons but they are not really representative of all kinds of neurons in the brain with a big fat axon and sparse dendrites. That's probably not true for 90% of neurons.

AD: This is a nice visual you draw here (Figure 3A). Tell me little bit about what comes to your mind when I say, "organelle movement along neurons"?

E: Right! This is when the microtubules come into picture. Say a spinal motor neuron that is almost close to a meter and we need a way to get materials from the cell body down to the terminal using the tracks along the axon.

AD: How will you represent your ideas about "organelle movement" in a visual format?

E: Draws figure 3. (Describing Figure 3A-B), let's assume cargo assembles in the soma after processing through ER and Golgi to package up and ready to go. Then the cargo is sorted to microtubules and kinesin. So we have microtubules bundles going down the axon and then the kinesin heavy chain help in transporting the cargo (could be organelles) across an axon in a neuron. Kinesin is a +end directed microtubule and so it takes cargo towards the neuron terminal. Several molecules get facilitated along the axon in this manner and so something of the size of an organelle can get transported like this too.

AD: Would mitochondria perhaps be in the picture (Figure 3B) anywhere?

E: Mitochondria could be an organelle that would be moved along. But I am not so sure of the size and I presume if it's too large, it might take a few kinesin molecules.

AD: How did scientists find out about the ideas you show in your figures (Figure 3A-B)?

E: Right. In terms of the organelle movement, probably through some form of live cell imaging and a fluorescent tag to tag some mitochondrial specific protein and track the fluorescence as it moves down the axon. The axons in the study obviously should be picked from the same kind of neurons, say spinal motor neurons, to avoid confounding factors that might contaminate our findings.

AD: How would be put that in form of a visual?

E: *(Draws Figure 3C-D)*

So in terms of materials we will have Mitochondria and GFP is the fluorescent protein tag specific to mitochondria that's coupled to the mitochondria gene. We will assume that's how it goes into the cell. Now we have GFP-tagged mitochondria and then we have microtubules which will be attached to kinesin. Basically then we will use a fluorescent microscope to track mitochondria.

AD: So in this experiment, would they be measuring something?

E: Yes! It depends on what they want to find out. If I were to assume let's say, my goal would be track the movement of the GFP labeled mitochondria (Figure 3C). Specifically we start measuring right around the axon hillock where the axon branches off (center image) and let's say we have a specifically identifiable particle for each mitochondria. We can then quantify the movement of the particles along a certain segment of axon observed under the microscope. So then in terms of measurement, we can measure position going from origin to end point of the imaging field and have time (in seconds) to track the movements over time (Figure 3D). I would then assign a value to each position a mitochondria (identifiable particle) is located at a certain

time and how many seconds does it take to reach a certain end point-so I will be measuring velocity in terms of quantity.

AD: Under what conditions would they made these measurements?

E: At this point hopefully we have neurons that are amenable to this procedure. So we will be using multiple neurons and then set up probably assigning sets of neurons in a randomized manner to several petri-dishes. Using the method I described, we can obtain several values for the speed of mitochondria moving towards an end point in the selected field which can be averaged eventually. I am guessing since we are only tracking movement in the neurons, a control won't be necessary at this point.

AD: Summary.

E: Our goal was to measure organelle movement within the axon. To do so, we fluorescently labeled particular organelle-mitochondria along the axon and then tracked its motion using live cell microscopy. We quantified those movements by looking at multiple sets of neurons to determine the positions of mitochondria and determined velocity and see whether there are different forms of movement.

Phase 2

Probe for surface-level reasoning

AD: Now! Here is a sheet with couple of figures that are the same figures you saw in the written survey you just completed. Along with these figures, here is another sheet with some background information. I would request you to take some time to go through these sheets. Let me know when you are ready. ***[Showed the figures 1a-c to the participant...gave them some time to think about what they are seeing…and followed up with these questions below...]***

E: [After couple of minutes] I think I am ready now…

AD: Great! So first, what are your thoughts about what's represented in the three figures [Referring to Figure 2a-c in the 'Neuron Assessment']

E: So these are showing a neuron and focusing on the axonal transport of mitochondria. There is also a enlarged version of the microtubule motors kinesin and dynein responsible for anterograde and retrograde transport respectively.

AD: What in this figure indicates you see a neuron?

E: The dendrites and an axon are typically parts of a neuron.

AD: What indicates you see transport of mitochondria like you just mentioned?

E: The arrows within Figure c tend to indicate motion.

AD: Where have you seen anything like this before?

E: Similar things in textbooks and in my own research.

AD: What the scientists trying to do in this study?

E: In this study there are trying to test the mechanism for a particular set of neurons that have impaired mitochondrial movement.

AD: What is their goal?

E: They want to figure out how to correct the impairment to be able to apply that to repair or preventing of neurons in patients with the disorder. They already are down to the idea that a defect with either kinesin or dynein is causing the disorder.

AD: Let's imagine you are the lead scientist of a group that is supposed address the goals that would just mentioned. What specific directions would you give your team to carry out this experiment using the materials provided? Also try maybe depicting it in some form of a visual like a schematic or flowchart.

E: So we will do a position vs. time of mitochondria and looking along the axons of neurons. We will have some control neurons taken from cell culture lines that basically don't show this impairment. Then we have the impaired neuron. What we expect to see then. Let me draw this out *(Draws figure 3E)*.

(Describing figure 3E) So we have a scenario 1: kinesin impaired and scenario 2: dynein impaired. Then we will have a control (normal neurons). When nothing is added, we get baseline for anterograde and retrograde speeds. With addition of compound K, we get retrograde movement only and with compound D, we will get a anterograde movement only. This will give us an estimate of the peak antero- and retrograde speeds and what to expect when we add something. *All others details were as tabulated in Figure 3E.* This is in the case where the impairment is assumed to be a loss of function.

AD: You mention "impaired" in this figure (Figure 3E). Where are the impaired neurons coming from?

E: These neurons are derived from the cell cultures of neurons of patients/cell lines with the impairment.

AD: How will you assign the treatments in the study?

E: In an ideal world, I would be blind as to the origin of the cell-so they wouldn't know whether the representative neurons are derived from the patient population or the normal human cell line. These cells will be randomly assigned to the three treatment groups which are my three columns (Figure 3E). So you will have nothing added first and do a series of measurements there and then you add the inhibitor compound and look to see the change over time.

AD: What is the rationale behind randomly assign the cells as you just mentioned?

E: It is a measure to reduce bias during the experiment and also to account for variability among measures.

AD: Why do you have multiple groups (Figure 3E)?

E: These are two sets of outcomes based whether the kinesin or dynein is impaired. It's useful to know what your predictions about an experiment would be so you can connect it back when interpreting results.

AD: So what were your predictions?

E: For scenario 1: With kinesin impaired neurons, I would expect the addition of compound K would show any change in the movement (because the impairment and inhibitor as the same impact). But with addition of compound D, I would see no movement in both the anterograde and retrograde directions along the axon.

AD: How will decide the right sample for the control vs. kinesin impaired vs. dynein impaired treatments (Figure 3E)?

E: Our target is the impaired mitochondrial movement. By having a positive control we know how the movements in a normal cell looks like. We also have an idea how the normal cell looks like when we have the inhibitors.

AD: What factors that you will specifically vary or keep the same in your experiment?

E: The factors kept the same would be the imaging set up, conditions of the medium, the cell culture age, time window used to measure, effective concentrations of the inhibitors *etc.* This ensures that any external sources of variation are removed in the experiment. Variation means the differences between measurements. The things we will vary are the treatments: nothing added, compound K or compound D.

AD: Let's say you perform the experimental approaches suggest, what kind of experimental results would you expect to get? How would you represent those findings?

E: First I would look at the baseline (Figure 3E, column 1) which could get us relatively far to understand whether the kinesin or dynein is impaired. Let's assume for convenience that our experimental with control group cells showed that dynein is impaired. So to represent how I reached upon that finding I would ideally draw a graph (Draws figure 3F).

So in a control cell from normal patients (Figure 3F, dashes), both anterograde and retrograde movement will take place towards the end point (100 μm). In the same kind of cell from normal patients, when compound D is added, we will notice anterograde movement only in the positive direction (dots). What we observe in the normal cells upon treatment with inhibitors can be then compared with the cells from the patients with the disease to test what we find in our study actually applies to the real patients.

So we might take a patient with the disorder, and because we know that most probably the patient has dynein impairment, when we add compound K (inhibits anterograde movement), we will see zero to no movement because both proteins are shut down- one by the disease and other by the inhibitor treatment.

The conclusion from this graph is that the dynein is impaired because in the control we see some proportion of retrograde motion but with dynein impaired we see only movement in the positive direction/anterograde movement.

In my graph, I am showing basically two groups because I focused on the different outcomes you control expect to get.

AD: How will you increase the validity of your experiment?

E: By doing that multiple times. Even though we think we have similar cells and conditions, there is going to be some variability between them and we want to determine the extent of variability.

AD: People sometimes talk about hypothesis-driven research. Your thoughts?

E: Its clearly something funding agencies prefer. It tends to drive how people frame questions. Up to a point it's useful but it's not necessarily how science was carried out a first few 100 years where it was done formally. I have some training in neuro-anatomy and it starts out more observationally and then from that you can start honing in on hypothesis but without a period of "fishing expedition", it's really hard to come up with more directive hypothesis. So one way could be you either retrospectively layout your hypothesis or have a clear starting hypothesis and are careful about your observations and let them allow you to refine your hypothesis.

Phase 3

AD: How would you rate these questions on a scale of 1-10? 10 being most comfortable and 1 being I hope I don't have to ever do this again.

E: I'd say 9 because its subject matter that I know a little bit about.

AD: Is there anything in particular about this question that you don't quite understand or find confusing?

E: Not necessarily. I know you did it to simplifying the context but I believe a large body of initial work would be required to get to narrowing down to kinesin or dynein being responsible for the disorder. So in a way I like that the question makes it easy by ruling out any other possibilities because just by itself, mitochondrial transport impairment could be potentially due to a host of things.

AD: Do you think any question about experiments is left out from what I asked you?

E: I guess there is the assumption that the experiment works in a straightforward manner. So an outcome wasn't given out. It was OK for me but for the students it would probably be not something they are used to because I don't think many come in already carrying some sort of knowledge about mitochondrial movement along neurons.

AD: if you were a diagram designer, would have drawn these pictures differently (Referring to Figure 2a-c in the question material)

E: The figures by themselves are quite okay. I know it doesn't include any measurement values because part of the question was for the students to think about that aspect. If you were to think about a classroom activity using this question, you would have the students go through the background information and perhaps sketch out plots and have that as supplement to the text.

AD: Do you think overall it's a good and clear question?

E: I think this is a fairly clear question. You can set up the experiment in a way that will give you some form of answer so it does lead you to derive a certain answer if you have the right ideas about designing an experiment. It leaves out a lot of aspects which is good because you can then question students about those like the things to measure and the logic/design of the experiment *etc*.

Even non experts who may be overwhelmed by some of the things here, between the figures and text they will probably do okay.

AD: What is general comment about participating in such exercises?

E: Depends on the frequency and time. I am fairly happy to participate in them. It's what I do on a regular basis.

2. Interview Transcript for Juan

AD: Interviewer; Juan (J): Student

Phase 1

AD: Hi! Juan, I am Annwesa Dasgupta (AD). How are you doing today?

J: Good!

AD: So, thank you for being here today. Alright, I would like to briefly explain some details about this activity. You just spent some time writing ideas about what you think about experiments. Now I would like to follow up your ideas by giving you an opportunity to share some thoughts verbally via a conversation based on a few questions. Are you ready to begin?

J:Yes!

AD: So, most of my questions will be related to the written survey you just completed, to help me understand your ideas. Some instructions to get started…please think freely about the questions I ask …there are no time limitations so you are free to take as much time as you wish to respond. There is no right or wrong answer to these questions. I am simply interested in your thinking about experiments. You are free to use provided materials to draw things if that helps you to express your thoughts. However, there might be certain instances where I request to visually present your ideas just so I am sure that I understand correctly. This interview will be recorded. You may choose to withdraw your participation at any time without penalty. If you have questions or need clarification at any time during this conversation, please let me know.

AD: let's talk a little bit about neurons. What's the first thought that cross your mind when I mention "neuron"?

J: like an axon and mitochondria.

AD: So then what do you think when I say "organelle movement in neurons"?

J: I know that kinesin and dynein controls the movement- as I saw in the written question. But I am not sure of what their functions were so…

AD: Before this question, what did you think of organelle movement within neurons?

J: not much-I never learned of it.

AD: Can you draw your ideas about neuron and organelle movement within it?

J: [starts drawing Figure 4A] so here's the axon. And the mitochondria goes from the cell body to the terminal which is controlled by kinesin and the other direction is controlled by dynein [Figure 2].

AD: So how did scientists' find out about what you depict in your figure [referring to Figure 2]?

J: through research and experiments.

AD: I see. So what kind of experiments would they have carried out?

J: They might have done individual experiments to find out about each part of this process. And then tried to see if one part is missing, what the effect would be on the process or how their role is necessary in the process.

AD: would they have made any measurement to figure out about the process?

J: they would be measuring the degree of necessity of a certain protein [kinesin or dynein] of the process. What is its function and if a part it needed for the body to continue functioning. If its removed what would be affected. Its specific role could be stopped or it might even stop roles of other parts too.

AD: You mention, they would have performed "individual experiments". Under what conditions would they have done these experiments?

J: they could remove kinesin and see that the mitochondria will only move one way which is probably a problem. Both the motor proteins might be necessary and their removal could lead to the disorder.

AD: would you please summarize your ideas about how scientists' would find out about the cause of a disorder with mitochondrial movement in neurons in 3-4 lines?

J: ok to summarize how scientists did their experiment, they would do individual experiments on the mitochondria, kinesin and dynein and see if they are needed. If they find that there is a problem with kinesin and/or dynein, they could manufacture genetically some substitute for the missing motor proteins and observe the effect.

Phase 2

Probe for surface-level reasoning

AD: Now! Here is a sheet with couple of figures that are the same figures you saw in the written survey you just completed. Along with these figures, here is another sheet with some background information. I would request you to take some time to go through these sheets. Let me know when you are ready. ***[Showed the figures to the participant...gave them some time to think about what they are seeing…and followed up with these questions below...]***

Juan (J): [After couple of minutes] Alright! I am ready now…

AD: Great! So first, what are your thoughts about what's represented in the three figures [Referring to Figure 2a-c in the 'Neuron Assessment']

J: This figure shows the axon and the mitochondria movement. It represents visually what kinesin and dynein functions are [refers to Figure 2b]. Figure c shows kind of an enlarged version of what goes on around this part of the axon.

AD: what indicates that you see an axon in this figure?

J: I know how a neuron looks and also same for an axon. But I have studied this process.

AD: What tells you that you see mitochondria moving?

J: Figure c shows and the text supplements information about anterograde and retrograde movement towards and away from the cell body with the help of kinesin and dynein.

AD: what are scientists trying to do in this study?

J: They are trying to study a disorder and improving it and seeing if a problem with kinesin or dynein is the cause of the disorder.

AD: What is goal for this study?

J: scientists want to see if kinesin or dynein malfunction is responsible in causing the disorder.

AD: How will they do that study?

J: They will set a control with all proteins in it and… [Pause]

AD: Would it help if you were to draw this out like a flowchart or a table?

J: Ok draws Figure 4C.

AD: how will you use the materials provided to design the experiment you just outlined in your figure [referring to Figure 4C]?

J: the scientists have a goal to find out does kinesin or dynein play a role in the cause of the disease. You can use compound K on neurons that lack kinesin as group 1 and use compound D on neurons that lack dynein as group 2.

AD: Why you suggest having multiple groups in your study as you show in your figure [refer to Figure 4C]

J: it's not one experiment-because you can't only see one group. You need like to verify your results.

AD: Tell me bit more about that idea?

J: like each group is assessing a certain compound or lack of a protein to see if only one protein is behind the disorder or both proteins have a role in the disorder. If you remove one with the patient improve?

AD: what would the right samples be for your control and group 1 and 2?

J: if you take out the neuron and place it in some atmosphere.

AD: let's say they decide use neurons as you suggest. Is there is a certain manner in which they will assign the neurons in the experiment?

J: they will select a patient with a disorder and one without the disorder and compare them and see what the differences are. And then do the experiment with neurons from patients with the disorder and use the one without the disorder as control.

AD: Based on that, what kind of results would the scientists get?

J: I predict that both proteins are necessary but the disorder patient is going to show a problem with the proteins in comparison to a patient without the disease. Maybe the disorder is that there is no anterograde movement because the mitochondria is not moving from the cell body to the cell terminal. Or in the opposite direction.

AD: Would they be measuring anything to reach to the results you suggest?

J: They'd be measuring movement of mitochondria. And they will see if the movement changes without the protein.

AD: How would you present these results?

J: my first, like, evidence would be from the imaging software in a table. A bar graph maybe…

AD: How would you draw that bar graph?

J: let's say he found that substituting kinesin with a genetically modified version has improved the disorder-makes the movement of mitochondria more effective. Then you can say movement with the disorder was this much and one without the disorder or the substituted version was normal and more effective [Draws Figure 4D]

Say, the second bar shows normal movement of mitochondria and the shaded bar is representing effectiveness mitochondrial movement in a person with the disorder of impaired mitochondrial movement so I am assuming there is no as effective movement.

AD: you show "effectiveness" as your y-axis. How will you measure effectiveness?

J: It will show how smooth the mitochondria moves. I am not sure what else to measure…

AD: In your table [refer to Figure 4C] you mentioned 2 groups and a control. Are you representing those in your graph [Figure 4D]?

J: this graph is for one group.

AD: So which group would this graph be for in your opinion?

J: I am not sure. I am just showing how the disorder will improve. I am not sure which group this would be for.

AD: summary!

J: I used compound K to remove kinesin and tested if that gave rise to the disorder. I would do the same thing with dynein. I will get the results but I don't know what they would be. But according to my example [refers to Figure 4C] when kinesin is lacking and thus, replaced with a genetically modified version of kinesin protein, the patient showed improvement in mitochondrial movement.

Phase 3

AD: How would you rate these questions on a scale of 1-10? 10 being most conformable and 1 being I hope I don't have to ever do this again.

J: I would say 5 because the questions were ok but the fact that almost everyone had to draw a visual, I didn't enjoy that.

AD: is there anything in particular about this question that you don't quite understand or find confusing?

J: [For the 'neuron assessment'] I thought that kinesin and dynein function should have been more clearly stated. If it is possible to remove them and yet not harm the patient!

AD: So from the information provided, the function of kinesin and dynein were not clear to you?

J: Well I know they are required for mitochondria to move in opposite directions in a neuron but I would like to know more about what is the problem with them that gives rise to the disorder. I would have like it to be clearer.

AD: Do you think any question about experiments is left out from what I asked you?

J: not off the top of my head

AD: if you were a diagram designer, would have drawn these pictures differently (Referring to Figure 2a-c in the provided question material)

J: yes! I would focus a little bit more on the two proteins and on the whole process of how the disease actually occurs in patients.

AD: What is your take answering such question in general?

J: like on an exam?

AD: Sure! But even during courses as study material?

J: Not very much.

AD: Tell me why?

J: well my opinion could be anything. I could predict any kind of information but I am not sure if I can get feedback on if it's correct or wrong. I am not ok with it! I like to know the right answer!

3. Interview Transcript for Eve

AD: Interviewer; Eve (E): Student

Phase 1

AD: Hi! ES *[name hidden for confidentiality]*, I am Annwesa Dasgupta (AD). How are you doing today?

Eve (E): Good!

AD: So, thank you for being here today. Alright, I would like to briefly explain some details about this activity. You just spent some time writing ideas about what you think about experiments. Now I would like to follow up your ideas by giving you an opportunity to share some thoughts verbally via a conversation based on a few questions. Are you ready to begin?

E: Yes!

AD: So, most of my questions will be related to the written survey you just completed, to help me understand your ideas. Some instructions to get started…please think freely about the questions I ask …there are no time limitations so you are free to take as much time as you wish to respond. There is no right or wrong answer to these questions. I am simply interested in your thinking about experiments. You are free to use provided materials to draw things if that helps you to express your thoughts. However, there might be certain instances where I request to visually present your ideas just so I am sure that I understand correctly. This interview will be recorded. You may choose to withdraw your participation at any time without penalty. If you have questions or need clarification at any time during this conversation, please let me know.

AD: let's talk a little bit about neurons. What's the first thought that cross your mind when I mention "neuron"?

E: Cells in your brain that have significant movement in your thinking process and anything that occurs in your body.

AD: Building on that, what comes to mind when I say "organelles moving in a neuron"?

E: specific organelles that take part in the processes needed to get neurons acting in the way they should or to produce the information they need throughout the body.

AD: That's interesting! How would you put these ideas in a drawing?

E: *Draws Figure 5A*

This is what I think. The cell is the neuron. I vaguely remember what it looks like because I took psychology so I kind of know the basis but since it's a neuron, it's going to be connected to other axons and it's going to distribute the information that going through. So there's the mitochondria and what's going on in the mitochondria determines how the transport occurs. So mitochondria

is going to give off the signals needed to the axon to go the other parts of the body to do whatever it was indicated to do.

AD: You draw this visual. Tell me how do scientists know what you are telling me?

E: I would assume that they have looked at quite a few brains probably through MRIs and CAT scans to see how the axons and neurons occur. They might have actually taken neurons from the brain and looked at them in a culture and see how they interact (Figure 5B).

AD: Ok. How would you put that in a drawing?

E: *Draws Figure 5B*

[Explaining Figure 5B] So through an MRI you notice areas that light up, so you could use substances that make certain areas light up under the MRI scan. An MRI might not be the best method because it's more of an outlook on the brain overall. If you want to see up-close you can then use a microscope and then you can see the cell.

AD: Great! Would they be measuring things here?

E: well you could see how the process occurs in the cell. They could watch as it happens. So they can then determine where the two proteins are present and watch as they occur.

AD: How would you specifically describe how they would have done those experiments?

E: well to be honest, I don't understand this completely as I haven't done the research. But with the basics, they would have to do things over a period of time-various experiments to compare. They would have to take a living specimen of the cells and keep it in the environment it needs to be so it functions properly. Then would watch as it occurs and inject what they need to manipulate things in the processes they observe to see what happens if they specifically change a certain thing- and how it affects the overall transport and other things.

AD: How would summarize your ideas about this experiment you proposed to discover organelle movement in neurons, in a couple of sentences?

E: well scientists are going to need to get a hold of these cells where they think a disorder is occurring and watch it as it happens. They have to get a significant amount of samples to test as they see fit. They are going to need the control which would be people that don't have the disorder. So healthy neurons and experiment would people that carry the unhealthy neurons.

AD: You mentioned, "A significant amount of samples". Tell me a bit more about that phrase.

E: I don't really know…they have to pick a number themselves but you need to the experiment multiple times and so you would have to have a decent amount of neurons from the healthy and unhealthy patients in order to conduct the experiment to compare and make sure that the results are significantly close to each other, otherwise the experiment really wouldn't be accurate. So it's not something you can just do once and expect to understand it. Multiple trials must be done.

AD: What is the value of doing multiple trials?

E: they get you further in the experiment-because if you just don't the study one time then you don't necessarily know how it's going to work differently. Since they wanted to test both motor proteins, you are going to have to test more than one anyway. You want to see how one affects it or how the other affects it or how both affect it. You can't really do all of that in a single trial. You would multiple trails for each of those and then you need to compare the end by taking averages.

Phase 2

Probe for surface-level reasoning

AD: Now! Here is a sheet with couple of figures that are the same figures you saw in the written survey you just completed. Along with these figures, here is another sheet with some background information. I would request you to take some time to go through these sheets. Let me know when you are ready. ***[Showed the figures to the participant...gave them some time to think about what they are seeing…and followed up with these questions below...]***

E: I am ready now…

AD: Great! So first, what are your thoughts about what's represented in the three figures ***[Referring to Figure 2a-c in the 'Neuron Assessment']***

ES: I think the diagrams show the basis of what the experiment is conducting. Figure c doesn't provide all the information it should. It's very minimal and basic. Figuresa-b are much more specific and they show where everything is located in respect to the cells. So I think they depict whatever they are supposed to depict more efficiently.

AD: So what's going on in these figures according to you?

E: the…um...the axon transports in anterograde and retrograde directions.

AD: what tells you that something is getting transported?

E: in the third figure the arrows indicate movement and the labeling anterograde and retrograde also confirm the movement. Unfortunately in Figuresa-b it doesn't exactly depict that. It just shows where the proteins are located in the cell.

AD: You mentioned the "axon transports". What indicates you see an axon?

E: Figure 2c is labeled axon.

AD: Where have you seen something like this before?

E: Not this exact process but in psychology I have seen similar types because you have to understand what neurons and axons work in the brain.

AD: So moving on the actual question, what are the scientists really trying to do here?

E: There are people with the disorder who are unable to perform transport that they need to and scientists believe that it has to do with the motor proteins-kinesin and dynein not working somehow and how that affects the movement of mitochondria

AD: What goal to these scientists have for this study then?

E: To determine if a problem with both, neither or one of the proteins *[kinesin and dynein]* is the source of the disorder and thus use that information to correct the process that is impaired in the disordered cells. So they want to fix that to make the neurons healthy in the person with the disorder to regain the movements that they need to carry out.

AD: So any idea how they would go about that?

E: the experiment?

AD: Sure. What would an experiment for this study involve?

E: Well you are going to need a control for an experiment [*Draws Figure 5C*]. The control will be the healthy neuron which has everything it needs to. Both neurons are going to contain the same organelles because that's required for the cell function. But experimental group will be the unhealthy neuron because we need to test that to find out about how the movement can be improved in the presence of kinesin and/or dynein. Control will just show the two proteins functioning normally.

AD: when you mention, "control and experimental group", what does that mean?

E: the control group is going to be everything you are in control of- so if you want a specific factor that you would like to maintain constant – that will be the control group. The experimental group is what you are going to add something to like the independent variable which you can decide how and how much of a variable is going to be added. Control is going to be set aside to see how things occur naturally and the experimental is you are going to decide how things occur.

AD: How would you use the materials provided in the study to actually perform your experiment?

E: the imaging software will help you record the movements that occur in the neurons. So you are going to use that for both control and experimental groups. The compound K and D are inhibitors which will be injected in the experimental groups to see how they affect the neurons. You may go about doing the experiments separately like trying, just one compound and then the other or both together.

AD: How would you visually represent the different experimental scientists might try?

E: *[Draws Figure 5D]*

So you can try cells with just kinesin inhibitor, just dynein inhibitor and then kinesin and dynein inhibitor together. And then neither of them. With compound K injected, you are going to record what happens. For dynein you would inject compound D into the cell. If you want to see how the two proteins interact, you are going to inject both compound K and D.

AD: Why do you show 4 experimental groups and one control group in Figure 5D?

E: because they mentioned two proteins. The proteins could interacting or acting separately. So one could have a hand in the process and the other couldn't or they could both be involved. They

want to see how the proteins work in the cell and they also want to try it without them just to see how the process would be affected without any proteins.

AD: How will you decide the right samples for you each of your groups (columns in Figure 5D)?

E: for the experimental since you are injecting the compounds, you can use the same type of cell but you would inject different compounds. The control you want to use the healthy neurons to see how the process works in general or on its own.

AD: How will you present results of this study?

E: I think the most efficient would be graph. If they want to convey all the groups then they could use a bar graph showing the amount of movement or how many movements for a specific time period.

AD: Let's try and draw that graph maybe?

E: for the control there will be just one bar graph [draws Figure 5E].

Unfortunately since I don't know which protein has the effect I won't...be able…to…

AD: So let's imagine that nobody really knows and you are the one who gets to be the first one to find this out.

E: (*Referring to Figure 5E)* I am going to assume that both proteins have a hand in the moving of mitochondria. So the control shows how the process should occur normally. With the [presence of] proteins individually, they might have a little bit of effect on mitochondrial movement. But with both inhibitors together, that is going to have movements most close to the control. The x-axis is the proteins themselves. So the bars in my graph show neurons with only functional kinesin (Graph b, bar 1), only functional dynein (Bar 2) and both functional proteins (Bar 3). And then the compounds are added to each kind of cell. I will then measure amount of mitochondrial movement with the imaging software although we don't have the healthy known amount of movements so you have assume that the control would provide the healthy amount of movements.

AD: So you think the control of a healthy neuron and healthy known amount of movements will be different in any manner?

E: I know it will be a little different in the unhealthy ones. So how the cells react is going to depends on how much you add, when and where you add it. Overall when you see movement for graph 2 (Figure 5E, right graph) closest to the control movement in graph 1 (Figure 5E, left graph), you would know that the experiment is successful.

AD: Tell me a little bit about you statement, "When you see movement for graph 2 closest to the control movement in graph 1, you would know that the experiment is successful."

E: The point of an experiment is to prove or disprove something to determine what you do is a success or a failure. Since we are saying that the control gives the healthy amount of mitochondrial movement needed, then with the experimental group you would want to find the

group which is most closely related to the healthy. So whichever one is closest of the healthy, would determine what solution you would use to help the disorder.

AD: Summary in couple of lines

E: I want to determine which protein helps in solving the disorder. You would need to set up control and experimental groups- you would lay this out for the scientists. I would tell suggests the scientists use the bar graphs to determine compare your results because you want to pick the protein that's producing movement similar to the control.

Phase 3

AD: How would you rate these questions on a scale of 1-10? 10 being most conformable and 1 being I hope I don't have to ever do this again.

E: Since I have a basic understanding of how this experiments work, so I might be around 5-10 depending on which experiment. I will be honest because the third one is the more difficult one, I could more sufficiently explain the first 2 question set.

AD: tell me why was the third one relatively difficult?

E: Since I don't know v. much about the process in general and it would work, I feel my lack of knowledge in this topic didn't help me when I was answering this question. But the first two questions were much easier to understand because you only needed to understand how the experiment works to explain the context confidently. But in this question I was very skeptical of my own answers just because I don't have all the background information I need.

AD: So you think that the background info and figures provided did not make it easy for you to answer this question?

E: The background does sum up the basics. But I am kind of person where I want to understand it more sufficiently in order to explain it to somebody else or in order to come up with an experiment in my own sense. It is very difficult to come up with an experiment if you don't understand what you are supposed to find out eventually.

AD: Do you think any question about experiments is left out from what I asked you?

E: No I think all aspects are basically covered. I would expect going into science, you would understand the experiments generally because they teach you the scientific method. Usually we don't have to come up with our own experiments because all information in terms of how you need to set it up is provided. But you have to understand the basis like the control and experimental groups etc. to get there.

AD: If you were a diagram designer, would have drawn these pictures differently (Referring to Figure 2a-c the provided material)

E: Figure c has the basics but you kind of want to see how it happens. It would be great if that could be demonstrated. Figuresa and b don't really show the process at all because it's just like here's everything in the neuron as its situated and here are the protein. So figures 2a-b really only

help with understanding the cell set up. Figure c gives information of how the process occurs but may be you could have given a lot more.

AD: Do you think is question is clear enough for you?

E: If you ever want to go into a science career, that you are going to have to be able to make your own experiments and understand how to set them up and how to analyze results. These three questions really make you think about that-because in all our previous experiences, we were told how to do the experiment! We didn't exactly have to come up with our own and this really pushes you to gain that knowledge you need to set up an experiment yourself!

AD: How do you feel about participating in such exercises about experiments?

E: I feel they should try to do something like this into the courses because if you are always given the experiment and how to do it, you are never going to understand how you would make your experiment. That could hinder how you would approach an experiment in your own lab later as a researcher. These make you think about it and seek the knowledge you need to understand, the process and how you would set up a typical experiment, what you need, how would need the control and experimental. What do you record? I feel they should do something like this in the courses.

AD: Great! Thank you for participating!

E: Thanks !

3. Interview Transcript for Li Na

AD: Interviewer; Li Na (L): Student

Phase 1

AD: Hi! ST *[name hidden for confidentiality]*, I am Annwesa Dasgupta (AD). How are you doing today?

Li Na (L): Good!

AD: So, thank you for being here today. Alright, I would like to briefly explain some details about this activity. You just spent some time writing ideas about what you think about experiments. Now I would like to follow up your ideas by giving you an opportunity to share some thoughts verbally via a conversation based on a few questions. Are you ready to begin?

L: Yes!

AD: So, most of my questions will be related to the written survey you just completed, to help me understand your ideas. Some instructions to get started…please think freely about the questions I ask …there are no time limitations so you are free to take as much time as you wish to respond. There is no right or wrong answer to these questions. I am simply interested in your thinking about experiments. You are free to use provided materials to draw things if that helps you to express your thoughts. However, there might be certain instances where I request to visually present your ideas just so I am sure that I understand correctly. This interview will be recorded. You may choose to withdraw your participation at any time without penalty. If you have questions or need clarification at any time during this conversation, please let me know.

AD: So let's start with telling me what you according to you is a neuron?

L : Neuron?

AD: Ya!

ST: I know that neurons transfer signals and if you get signal from outside of the body like someone touches you or you hear something, the neuron can transmit that information to your brain.

AD: How would share that in a drawing? Also please label your diagram.

L**:** *Draws 6A*

AD: This is a nice drawing (referring to Figure 6A). This is your drawing about neurons. Now if I ask you what you think about "organelles moving inside of neurons", what would you say?

L: before this survey I just knew about how neurons communicate with each other and how the gradual change in ions across a membrane help in transmitting signals along axons (as drawn in Figure 6A). I only know about this aspect but I don't know anything about mitochondria transportation.

AD: Ok you drew this figure of a neuron. Can you picture mitochondria in the neuron anywhere?

L: maybe just along the axon (Draws and labels *mitochondria* in Figure 6A).

AD: how did scientists discover the ideas you share in your nicely drawn Figure 6A?

Li Na: They might have labeled the important organelles.

AD: So you mention "labeling". Tell me a little more about that?

L: maybe somehow they would amplify the process and label some important organelles. They could explain it in words instead of drawing it because they might not know how the process looks.

AD: Would they have made any measurements?

L: So if we consider that scientists know the structure of organelles but they are not sure how they move, they could measure the direction and displacement or electrical potential.

AD: Under what conditions would they have made these measurements?

L: Might have labeled the important organelles. Also the presence of different amounts of ATP present might affect the directions in which the organelles move.

AD: Any idea how they would they have actually carried out what you suggest?

L: They would have to use a computer program because they organelles are really small. I don't think you can they can be recorded using naked eye.

AD: How would summarize your ideas in a couple of sentences to explain your ideas on what scientists would do to measure movements along a neuron?

L: I don't know how to explain it. Let me try. I would first set up a hypothesis.

AD: What would that hypothesis be then?

L: The scientists want to measure which organelle will cause movement in different directions. After the hypothesis, they will set up an experiment.

AD: How would they go about that?

L: they know how the organelles move but they don't know *[pause]*…they know the structures and the movement are based on myosin. They consider other variables that would cause a difference in the direction of movement.

AD: When you mention variables, what do you mean?

L: You need to change certain things and not just observe them. After that, you get different responses from variables in an experiment.

Phase 2

Probe for surface-level reasoning

AD: Now! Here is a sheet with couple of figures that are the same figures you saw in the written survey you just completed. Along with these figures, here is another sheet with some background information. I would request you to take some time to go through these sheets. Let me know when you are ready. ***[Showed the figures to the participant...gave them some time to think about what they are seeing…and followed up with these questions below...]***

L: *[after few minutes]*... I am ready. I just went through these sometime back so I am familiar with these.

AD: What are your thoughts about what's represented in this diagram?

L: In Figure 2a, I know that the mitochondria are along the axon of a neuron and I can compare Figure 2a and 2c. I find Figure 2c an easy one. I also see a cell nucleus and cell body. Figure 2c is more easily understandable but the other one gives a more accurate structure. Figure 2b is an amplification of Figure 2a.

AD: So what's going on in these figures?

L: I know the kinesin and dynein can cause movement in different directions of mitochondria because I see arrows in Figure 2c which tells me about a difference in directions. Figure b is really different. I see microtubules around the mitochondria but in Figure a I don't really see microtubules. I also notice that a difference in calcium ions cause a difference in direction. So ions interaction causes a difference in direction.

AD: so you mentioned this is 'neuron'. What do you think so?

L: from the structure in Figure 2a which is really representative of a neuron.

AD: what features of a neuron do you see here?

L: different terminals like cell terminal and there is a cell body.

AD: Where have you seen a neuron before?

L: just in the textbook from my course before.

AD: you mentioned about "movement in different directions"? What tells you that you see movement?

L: I see myosin and ATP which I guessed indicates an energy change and movement.

AD: What are scientists trying to do in this study?

L: they are trying to the find the cause of a disorder.

AD: Tell me a little more about that.

L: The disorder may bring pain to the patients so they are trying to find a way to cure them. The transportation in the anterograde and retrograde directions are both activated because kinesin and dynein are both active. So the mitochondria cannot move in either direction because the kinesin and dynein cancel each other-and so this maybe the disorder.

AD: How would they use the materials provided to study the cause of the disorder as you just mentioned?

L: they might try four combinations (outlines in Figure 5B) as treatments for the mitochondria.

AD: When you mention treatments, what do you mean?

L: Treatment… *[pause]*..Before the treatments the subjects should have the same conditions and then you try different things on them and see the response.

AD: Tell me more about what you mean when you say, "Before the treatments the subjects should have the same conditions"?

L: if they don't have the same conditions, they may react differently and that may lead us to think about false causation.

AD: So what kind of conditions would you keep the same in this study you are proposing?

L: I will keep the same organelles under observation, use the same species of organisms for the neurons and use cells from the same one animal. And also make sure that they are in the same environment.

AD: So you mention 4 combinations? Why so?

L: for an experiment, they need to find a cause and for that they need to set up control groups and experimental groups. We are given two compounds, a kinesin and a dynein inhibitor and by inhibiting we can look for effect on neuron function.

AD: What does a control group mean to you?

L: The baseline. I cannot remember the exact concept. But you need a control group to come the experimental groups to it.

AD: how will you decide the right sample for the treatment and control group?

L: the sample/subject is the mitochondria in the neuron and kinesin/dynein is the variable because they will be either inhibited or activated. If in the control group, displacement of mitochondria in either direction is zero.

AD: What kind of results do the scientists expect to get from the combinations you suggest?

L: the kinesin moves mitochondria in the anterograde direction while dynein moves it in the retrograde direction. Both if activated together will result in the disorder. Then I will measure the direction and displacement and draw a graph like this (*Draws Figure 6C*).The y-axis will show

the displacement and x-axis shows "+" for anterograde movement and "-" for retrograde movement. Group 1 is the control group. Group is activated kinesin and inhibited dynein so we see only anterograde movement. Group 3 is both activated. Group 4 dynein active and kinesin inhibited so the movement is in retrograde direction.

AD: In what format will the results be recorded?

L: I think the results should be recorded in form of numbers. Maybe displacement can be measured in terms of length in micrometers.

AD: If you had to go back and summarize the overall experiment you designed from beginning to end in a couple of sentences, what would you say? If it helps you can also visually represent your experimental proposal.

L: First I would have a hypothesis. Then do the experiments. Then show the results. When kinesin is activated and dynein is inhibited, we see movement in the anterograde direction. When dynein is working and kinesin is inhibited we see movement in the retrograde direction. When both are activated, the functions of the two proteins are replicated and thus, the mitochondria cannot move in either direction so the movement is impaired.

AD: You mentioned replication. What does replication mean?

L: when a large number of samples are used to avoid the chance variable.

AD: What is a 'chance variable'?

L: I have just learned this few weeks ago. Having small groups might lead us with results that are not persuasive. If you get a larger number of samples, you can see the outliers of the data clearly and then just pick the values that lie centrally.

AD: How would you increase the validity of your experiment?

L: by using randomization. When you choose the samples, you assign them randomly.

AD: Describe that a little more.

L: cells even when taken from one animal might have differences. So when you extract them you need to pick them randomly and then also randomly assign them to the experimental groups. People might do that to decrease the confounding variables-so if one group has a special tendency for a certain kind of trait; they will react and lead us to wrong causation. So randomization is very important.

Phase 3

AD: How would you rate these questions on a scale of 1-10? 10 being most conformable and 1 being I hope I don't have to ever do this again.

L: I would say 9.

AD: Tell me why?

L: I think I can come up with a lot of ideas so I am comfortable with activities like this.

AD: Is there anything in particular about this question that you don't quite understand or find confusing?

L: yes. In Figure 2b, I see calcium ions but I am confused about the roles of that.

AD: Do you think any question about experiments is left out from what I asked you?

L: yes! How are the kinesin activated or inhibited? What causes their activation or inhibition? Most of the people usually don't carry this disorder so one functions then…but I think both are present in neurons structurally. But how can they be selectively activated or inhibited? I am not sure how the compounds cancel each other.

AD: if you were a diagram designer, would have drawn these pictures differently (Referring to Figure 2a-c in the question material)

L: I am confused about how the mitochondria are outside the microtubule. Also I will label ions for dynein.

AD: Do you think is question is clear enough for you?

L: Maybe. I don't know the answer to this experiment so whether the question is good depends on the answer.

AD: How do you feel about participating in such activities about experiments?

L: Maybe it's good for future. I find it interesting!

4. Interview transcript for Daniel

Interviewer: AD Student: Daniel

Phase 1

AD: Hi! DW [name hidden for confidentiality], I am Annwesa Dasgupta (AD). How are you doing today?

Daniel (D): Good!

AD: So, thank you for being here today. Alright, I would like to briefly explain some details about this activity. You just spent some time writing ideas about what you think about experiments. Now I would like to follow up your ideas by giving you an opportunity to share some thoughts verbally via a conversation based on a few questions. Are you ready to begin?

D: Yes!

AD: So, most of my questions will be related to the written survey you just completed, to help me understand your ideas. Some instructions to get started…please think freely about the questions I ask …there are no time limitations so you are free to take as much time as you wish to respond. There is no right or wrong answer to these questions. I am simply interested in your thinking about experiments. You are free to use provided materials to draw things if that helps you to express your thoughts. However, there might be certain instances where I request to visually present your ideas just so I am sure that I understand correctly. This interview will be recorded. You may choose to withdraw your participation at any time without penalty. If you have questions or need clarification at any time during this conversation, please let me know.

AD: let's talk a little bit about neurons. What's the first thought that cross your mind when I mention "neuron"?

D: like nerves.

AD: tell me a bit more about that…

D: Just like signals throughout your body-signals to move or other processes.

AD: If you had to draw a nerve, what you would draw?

D: something like...I guess [Draws Figure 7A] a tree. So you start with a thicker nerve and then it branches off, into smaller and smaller pieces, until it gets to the end…

AD: Would you label any parts?

D: I don't really have anything to label.

AD: Ok! Building on this figure of a neuron, when I say organelles moving along neurons, what would you say?

D: uhh…I don't know I just think of electrical signals. Other than that I don't have any information.

AD: you mentioned, "Electrical signals". How would you depict that in this figure?

D: Umm I don't know. I would assume it would move against the wall of the neuron [Figure 7A].

AD: How did the scientists' find out about the things like electrical signals along neurons etc.?

D: I would assume some sort of experiment involving people with impaired nerves or something along that nature. Then comparing that to like a control group with others that have normal/regular nervous system.

AD: How would you schematically depict what you just mentioned?

D: [*Draws* Figure 7B] So you have a control carrying people whose nervous system isn't impaired. Then you would have to compare signals among people in the control groups with people in the experimental group that have an impaired nervous system.

AD: When comparing signals [Figure 7B], would the scientists' be measuring something?

D: I am sure they would be measuring something because they probably should be something that could be measured. You could measure the strength of the electrical signals or the path the signal takes and see differences in the way a normal person's body would send signals out vs. somebody with an impaired nervous system. And how the body responds…

AD: Would there be any numbers involved?

D: If that's possible. That's probably the best way to do it. But I am not sure…

AD: Under what conditions would they be making these measurements?

D: they would probably have two similar types of people with as little different between them except for the nervous system.

AD: Why do you suggest that?

D: people that are of different height would either send weaker/stronger signals because of the distance they would have to travel. Age might affect it. So the two types of people should be very similar except their nervous system.

AD: You mentioned great ideas to suggest what scientists would have probably done to find out about electrical signaling along neurons. How would you summarize in 3-4 short sentences?

D: scientists would try to measure the electrical signals in the two different groups: 1) control group with normal nervous system. 2) Another group that would have the nervous system impaired in some way and they would compare the signals/path/strength or something like that in the two groups. They would try to keep those as similar as possible so it's just the nervous system that's different between the two so the results aren't affected.

AD: Results aren't affected means what?

D: I mean if there is a difference between heights of subjects in two different groups, you wouldn't be able to necessarily decide if it was the height that gave rise to the difference in strength of the electrical signals rather than the nervous system.

Phase 2

Probe for surface-level reasoning

AD: Now! Here is a sheet with couple of figures that are the same figures you saw in the written survey you just completed. Along with these figures, here is another sheet with some background information. I would request you to take some time to go through these sheets. Let me know when you are ready. ***[Showed the figures to the participant...gave them some time to think about what they are seeing…and followed up with these questions below...]***

D: [After couple of minutes] I think I am ready now…

AD: Great! So first, what are your thoughts about what's represented in the three figures [Referring to Figure 2a-c in the 'Neuron Assessment']

D: the mitochondria moves through the axon in Figure a, which sends some sort of signal and then its moved using the two proteins [kinesin and dynein].

AD: You mentioned that mitochondria moves? What in the figures gives you an indication of movement?

D: I'd say the arrows on Figure c show that one protein goes one way and the other goes the other way. They move along an axon of a neuron.

AD: What tells you that you see a neuron?

D: I don't know. I think just because it said in the part of the question. But it also kind of looks like what I drew earlier so I think I am familiar with a similar structure of the neuron.

AD: Cool! Have you seen figures like this before?

D: I don't know about this stuff specifically but I know like biology classes in high school they have shown more basic figures of what nerves looks like without the more detailed explanation.

AD: What are scientists' trying to do in this study?

D: they think the two proteins help in the movement and some disorder is caused they believe by the proteins not doing what they are supposed to. This causes the mitochondria to not move how it should. They are trying to determine first of all, if actually these are the proteins that help

movement and then want to determine if those are what's wrong with people who have the disorder.

AD: So do they have a goal in this study?

D: to find out which of the two proteins causes the disorder so that they could try to fix it?

AD: What ideas do they have in terms of that goal?

D: They have two different compounds to inhibit the two different proteins and observe which inhibited protein affects mitochondrial movement in a manner similar to the movement in people with disorder. They also have software to measure the movement with those who has the protein inhibited or when they are not. They can use the imaging software and determine the movement with the inhibited proteins and see if it's similar to the movement in those who have the disorder.

AD: Let's imagine you are the lead scientist of a group that is supposed to conduct the experiment you just described. What specific directions would you give your team to carry out this experiment using the materials provided? Also try maybe depicting it in some form of a visual like a schematic or flowchart.

D: Ok it might be easier for me to think about it and draw something first.

AD: Sure go ahead; take your time to draw ideas.

D: [Draws Figure 7C]

AD: Can you please walk me through your diagram [Figure 7C]?

D: Ok so I started out with measuring movement of mitochondria in nerves of a normal person. Then I split a group of normal people's cell cultures into four different groups, control groups, one with compound K, one compound D and one with both. I am assuming these people were similar to each other as much as possible, in like their health conditions, such that we know that the observed effect is due to the application of compound K or D. Then you could measure the movement in each of those groups. Then compare the movement with multiple patients who have the disorder with the 4 groups of patient. This will allow us to infer that those were the protein that caused the disorder.

AD: What does a control mean to you in an experiment?

D: I guess a group that would not be receiving any treatment but other than that it would still be subjected to the same conditions as those who are given the treatment (compounds in the case of this study).

AD: Tell me why do you have 4 groups here (Figure 7C)?

D: The control group allows them to measure changes in the movement while the experiment was going on. Just the K and D because those are two things whose effect will be measured. I figured I would test both in case the patient had both that weren't working correctly. Then you would also have to have the group of patients [with the disorder] to be able to test to see if the

difference in their movement was the same. So they would know what they found in their experiment is actually what is wrong with the patient.

AD: how will you decide what kind of patients participate in your control vs. other groups with compounds applied?

D: I would randomly assign them into groups. Like I would number each patient and use a random no. generator…so for example, if this was out of a 100 people, the first 25 are placed in the control, the second 25 in the next group and so on….

AD: What is the relevance of "randomly assigning" as you mention?

D: if you just grouped them in a non-random manner it wouldn't be evenly spread out between all the different variables. If you did it by height, you would bias the results and find differences across groups due to the height differences rather than a result of compound application.

AD: What kind of results do scientists expect to get? What would those mean?

D: Like before I will try drawing it out *[Draws Figure 7D]*.

So…I just made up different numbers they might have gotten as results although I am not sure of the units on it. Then just take the patient with disorder and if it matched around the same range as movement in the compound D, they would know that a problem with dynein is the cause of the impaired mitochondrial movement.

If it was a different number, they would know a problem with those compounds have no role to play in causing the disorder.

AD: How would scientists visually represent these results? How would they communicate their results to another group of scientists?

D: They would probably present a report with graphs.

AD: How would they draw that graph?

D: *[Further adds to Figure 7D]*

AD: How would you explain this graph to me?

D: I listed the different treatments on the x-axis. Along the y-axis is the movement compared to the control group. I would just graph the difference in movement from one to the other. Then you would compare to see how similar are the differences with the treated cells to the actual cells from patients with the disorder.

So the first bar shows that with treatment with compound K, the mitochondria moved 4 units less than the control groups it over a specific period of time. And so because treatment with compound D moved 6 UNITS less than the control group, dynein inhibition more strongly affects overall mitochondrial movement. Alternatively, you could also just graph a bar for the control group and compare them.

The scientists could then develop something to make the protein work or fix the existing problem somehow.

AD: How would summarize your experiment in 3-4 lines?

D: 1. Measure movement of mitochondria in neurons for a group of randomly picked normal persons who are as similar to each other as possible in terms of general health conditions.

2. Split cells of normal persons into 5 different groups. Each group carries a different treatment as outlined in the Figure [normal person; control with no treatment, one with compound K and another one with compound D; one gets both]

3. Compare your movement with the treatment groups to the movement in neurons of a patient with disorder to see if there are any similarities in trends of the movement. If they did have the same movement, you could argue the source of the disorder as per your treatment.

Phase 3

AD: How would you rate these questions on a scale of 1-10? 10 being most conformable and 1 being I hope I don't have to ever do this again.

D: I was pretty comfortable with the way the questions were framed so I would say 9.Only thing I wasn't so sure about was not knowing more background information when designing experiments or answering questions. Just not being sure what exactly might be affected in the real patients.

AD: is there anything in particular about this question that you don't quite understand or find confusing?

D: The only thing I found confusing was Figure 2b which was little busy.

AD: Did the diagram and background information, help you, in thinking about your ideas?

D: The diagrams definitely helped me think about the process more clearly since I did not know about this process too much before this study. I think it helped me see how things like the mitochondria, kinesin, and dynein are placed within a neuron.

AD: Do you think any question about experiments is left out from what I asked you?

D: I don't think so….

AD: If you were a diagram designer, would have drawn these pictures differently (Referring to Figure 2a-c)

DW: I don't know about changing them but most textbooks have a couple of sentences explain each figure. Including something like that might be helpful to better understand the process of what's going on.

AD: Overall do you think this is a clear question?

D: yea it was pretty good. I like it. After reading all the provided material it was easy to understand what information they already had and what they are not looking for.

AD: What is your take answering such question in general? How do you like the process of figuring out about experiments in a format that you just participated in?

D: I liked it! It was quite okay. So far in biology we haven't really had to come up with our own experiments. It's more of we were asked to read what other people had done and their experiments and how they dealt with different things. It's nice and probably important to be able to think through what you would do as a scientist. This pushes me to decide about things I haven't thought of before.

Supplement F Table 2: Experts' reasoning with visualizations (RM) before and with the 'Neuron Assessment'

Concepts	**RM***	**Before**	**With**
		Neuron subject matter	
a. Neuron knowledge	**Spatially manipulate a representation** (Figure 3A)#	Spatially manipulate a representation (figure of a neuron) to interpret and explain a concept (neuron anatomy).	
	Visualize levels of organization	Visualize levels of organization, relative size and scale (relative size and shapes of cell body, axon and mitochondria).	
	Decode a representation		Decode the symbolic language composing a representation (Figure 2a-c)
	Translate horizontally across representations		Translate horizontally across multiple ERs of organelle movement in neurons (Figure 2a-c).
b. Organelle movement in neurons	**Interpret temporal resolution**	Temporal resolution of steps in cargo transportation along microtubules during cellular processes of vesicular/organelle transport across neurons (Figure 3 B)	Temporal resolution of mitochondria movement along neurons – position of organelle along axon over time (Figure 3F).
	Translate horizontally across representations	Translate horizontallyacross multiple ERs of a concept (multiple figures representing various aspects of organelle movement) (Figure 3B).	Translate horizontally across multiple representations of neurons (Figure 3E).
		RED areas	
c. Experimental design representations	**Interpret and use a representation**	Provided neuron figures were interpreted to demonstrate design of an observational experiment involving GFP labeled tracking of mitochondria (Figure 3C).	Provided neuron visuals were interpreted to design experimental groups and solve a problem of investigation of organelle movement in neurons (Figure 3F).

* RM refers to Reasoning about the symbols and Modes of representations listed in Supplement Table 1

θ Superscripts refer to the concepts listed in column 1 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php

Supplement F Table 2: Experts' reasoning with visualizations (RM) before and with the 'Neuron Assessment'

Concepts	RM*	Before	With
Control group[20] Treatment group[18]	**Construct a representation**	The representation suggests an observational experiment (GFP labeled tracking of mitochondrial movement along neurons) but no experimental groups were identified.	The representation represents manipulation of control and treatment variables organized as separate groups in a table (Figure 3F) [2, 18].
d. Graphs RED areas: Variable[20] property of an experimental subject Manipulation of variables[17] Measurement of outcome[70] Accounting for variability[22] Scope of inference[15]	**Interpret and use a representation** (Figure 3C)	Provided neuron figures were interpreted to demonstrate design of an observational experiment involving GFP labeled tracking of mitochondria.	
	Construct a representation	Graph constructed to represent findings from the observational experiment) (Figure 3D) [3, 5]. Graph constructed with independent variables and dependent variables on x-axis and y-axis respectively. Specific treatments are represented as curves. Dotted line present outliers from variation (Figure 3D) [2, 3, 4].	Graph constructed to represent control and treatment variables organized as separate curves) (Figure 3F) [1, 2]. Graph constructed with independent variables and dependent variables on x and y-axes[2]. Different treatments are represented as separate points. Dotted line present outliers from variation[4] (Figure 3F).
	Translate horizontally across representations	Horizontal translation across multiple representation of an observational experiment tracking movement of mitochondria along axons (Figure 3C-D).	Horizontal translation across experimental table and experimental graph representing each treatment in the table as separate curves on the graph appropriately (Figure 3E-F).
	Interpret the temporal resolution of representations	Movement of organelle along neurons– position of organelle along axon over timedepicted[3] (Figure 3D).	Movement of organelle along neurons– position of organelle along axon over time depicted (Figure 3F).

Correct ideas Difficulties Lack of evidence

[θ] Superscripts refer to the concepts listed in column 1 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
		Neuron subject matter			
(I) Neuron concepts	a. Neuron knowledge	i. *"[In a neuron] there would be dendrites, an axon which can be myelinated, circular soma and some dendritic branches going up."*	Memorize entities: axon, dendrites, myelination, soma	ii. *The dendrites and an axon are typically parts of a neuron.*	Memorize entities: axon, dendrites
	b. Organelle movement	i. *"[In organelle movement] the cargo is sorted to microtubules and kinesin. So we have microtubules bundles going down the axon and then the kinesin heavy chain help in transporting the cargo (could be organelles) across an axon in a neuron."*	Apply knowledge of concepts (molecules like kinesin, microtubules, kinesin heavy chain) to explain organelle movement	ii. *"In this study there are trying to test the mechanism for a particular set of neurons with impaired mitochondrial movement, to figure out how to correct the impairment and apply that to repair or preventing of neurons in patients with the disorder. They are already down to the idea that a defect with either kinesin or dynein is causing the disorder."*	Apply knowledge of concepts (neurons, molecules like kinesin, microtubules, dynein) to explain investigation goal of diagnosing impaired mitochondrial movement.
		RED areas			

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	Before (Phase 1)	RC	With (Phase 2)	RC
(1) Variable property of experimental subject	a. Experimental subject *Sample*[13#] *Subject*[16] *Unit*[19] *Variable*[20]	i. *"We have GFP-tagged mitochondria*[16] *and then we have microtubules*[16] *which will be attached to kinesin. Basically then we will use a fluorescent microscope to track (moving*[20]*) mitochondria*[16]*."*	Integrate knowledge of concepts (mitochondria, microtubules, kinesin, fluorescent microscope) with experimental subject[16] and its variable[20] property i.e. movement.	ii. *"We will do a position vs. time*[20] *of mitochondria and looking along the axons of neurons*[16]*. We will use neurons are derived from the cell cultures of neurons*[16] *of patients/cell lines with the impairment*[13]*. There will be scenario one with kinesin impaired and scenario two with dynein impaired neurons*[19]*"*	Apply knowledge of concepts (neuron cell cultures) to propose an experimental subject[16]along with a variable[20] property (impairment).
(2) Manipulation of variables	a. Treatment variable *Subject*[16#] *Variable*[20] *Treatment variable*[17] *Treatment group*[18]	i. *"Using live cell imaging and a fluorescent tag to tag some mitochondrial specific protein and track fluorescence as it moves down the axon."*	Lack of Evidence	ii. *"To each of these kinesin impaired and dynein impaired cell lines*[18]*. I will add compound K, compound D respectively as treatments*[17]*"*	Transfer and apply knowledge of variable[20] property of the experimental subject[16] (kinesin/dynein impaired neurons) to propose treatment (independent) variables[17] (compound K/compound D).

[#] Superscripts refer to the concepts listed in column 2 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php

[#] Superscripts refer to the concepts listed in column 2 and defined in Supplement A

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
	b. Control variable *Control*[1] *Control group*[2]	i. *"I am guessing since we are only tracking movement in the neurons, a control [1, 2)] won't be necessary at this point."*	Lack of Evidence	ii. *"We will have a control (normal neurons*[1]*). When nothing is added, we get baseline for anterograde/retrograde speed. To a group of normal neurons we will add compound K and D respectively.*[2]*"*	Transfer and apply knowledge of the concept of control[1, 2] for comparison purposes.
	c. Controlling outside variables *Confounding variables*[8] *Control group*[2] *Treatment group*[18] *Variation*[21]	i. *"The axons in the study obviously should be picked from the same kind of neurons*[21] *to avoid confounding factors*[8] *that might contaminate our findings."*	Apply knowledge of ways to reduce variation[21] by controlling confounding variables[8].	ii. *"The factors [across treatment*[18] *and control group]*[2] *kept the same would be the imaging set up, conditions of the medium, the cell culture age, time window used to measure, effective concentrations of the inhibitors etc*[8]*. This ensures that any external sources of variation*[21]*n are removed in the experiment."*	Apply knowledge of matching treatment[18] and control group[2] variables to propose ways to deal with variation[21] from confounding variables[8].
(3) Measurement of outcome	a. OutcomeVariable[20] *Subject*[16] *Outcome variable*[7]	i. *"We then quantify the movement of the particle*[7, 16, 20] *along a certain segment of axon."*	Apply knowledge of variable[20] property of experimental subject[16] under investigation to propose measureable outcome variables	ii. *"So in a control cell from normal patients, both anterograde and retrograde movement will take place towards the end point (100 µm). In the same kind of cell*	Apply knowledge of variable property of experimental subject[16] (anterograde/retrograde movement)

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	Before (Phase 1)	RC	With (Phase 2)	RC
			(movement of particles). Reason locally about outcome variables[7] (movement of particles along the axon).	*from normal patients, when compound D is added, we will notice anterograde movement only in the positive direction (100 µm)* [7]. *What we observe in the normal cells upon treatment with inhibitors can be then compared with the cells from the patients with the disease to test what we find in our study actually applies to the real patients."*	under investigation to propose measureable outcome variables[7] (movement of particles).
(4) Accounting for variability	a. Replication[1] [2#] *Variability*[22] *Subjects* [16] *Units*[19] *Treatment group* [18] *Control group*[2]	i. *"We will be using multiple neurons*[16, 19] *and using the method I described, we can obtain several values*[12] *for the speed of mitochondria moving towards an end point in the selected field which can be averaged*[22] *eventually."*	Apply knowledge of ways to reduce variability[22] from experimental subjects[16] or units[19] by averaging values as a result of replication[12].	ii. *"We would take measurements [for the treatment and control groups] multiple times*[18]. *Even though we think we have similar cells* [16, 19] *and conditions, there is going to be some variability*[22] *between them and we want to determine the extent of variability*[16]*"*	Apply knowledge of ways to measure and reduce variability[22] by replicating[12] measurements on multiple cells[16, 19] in treatment[18] and control groups[2].
	b.	i. *"We will be using multiple*	Apply knowledge of	ii. *"Randomly assigning*	Apply knowledge

[#] Superscripts refer to the concepts listed in column 2 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
	Randomization[1] *Random sample [10] Treatment groups [18], Variability [22]*	*neurons picked randomly[10] and then set up probably assigning sets of neurons[18] in a randomized manner[11] to several petri-dishes."*	ways to reduce variation[22] by randomized assignment[11] of treatments[18].	*[11] cells [of blind origin] [10] to 3 [treatment] groups [18] reduces bias during the experiment and accounts for variability among measures[22]"*	of ways to reduce variability[22] by selecting a random sample[10] and by randomization[11] of treatments[18].
	c. Representative sample[10] *Sample [13] Random sample [10] Control group[2] Treatment group[18]*	i. *"Often in textbook, the spinal motor neurons are shown as the representative neurons[10] but they are not really representative of all kinds of neurons in the brain with a big fat axon and sparse dendrites[13]. That's probably not true for 90% of neurons."*	Memorize knowledge of spinal motor neurons[13]. Apply knowledge of representative sample [10, 13](of neurons) as measure to account for variation.	ii. *"I would be blind as to the origin of the cell [10, 13]-so they wouldn't know whether the representative neurons are derived from the patient population (treatment group) [18] or the normal human cell line (control group)[2]"*	Transfer and apply knowledge of representative sample[10] (of neurons) to sample[13] of experimental subjects as part of treatment group [18].
(5) Scope of inference	a. Scope of Inference[15#]	*i. Our goal was to measure organelle movement within the axon. We fluorescently labeled particular organelle-mitochondria along the axon and then tracked its motion using live cell microscopy. We quantified those movements by looking at multiple sets of neurons to determine the positions of mitochondria and*	Lack of evidence	ii. *"What we observe in the normal cells upon treatment with inhibitors can be then compared with the cells from the patients with the disease to test what we find in our study actually applies to the real patients[15]"*	Reason locally and globally about scope of inference[15] to make conclusions about an investigation.

Supplement F Table 3:Experts' reasoning with experimental design concepts (RC) before and with 'Neuron Assessment'.

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
		determined velocity and see whether there are different forms of movement.			
	b. Cause and effect[4] *Treatment variable [17] Control variable[1] Outcome variable [7] Correlations[3]*			ii. *"We might take a patient with the disorder[17], and because we know that most probably the patient has dynein impairment, when we add compound K (inhibits anterograde movement), we will see zero to no movement."[7] "The conclusion from this graph is that the dynein is impaired because in the control we see some proportion of retrograde motion but with dynein impaired we see only movement in the positive direction/anterograde movement."[3,4]*	Apply knowledge of treatment[17], control[1] and outcome[7] variables to develop causal[4] explanations.

Supplement F Table 4:Juan's reasoning with visualizations (RM) before and with 'Neuron Assessment'

Concepts	**RM***	**Before**	**With**
Neuron subject matter			

* RM refers to Reasoning about the symbols and Modes of representations listed in Table 1

Supplement F Table 4:Juan's reasoning with visualizations (RM) before and with 'Neuron Assessment'			
a. Neuron knowledge	**Spatially manipulate a representation**	A neuron is spatially manipulated to explain neuronal anatomy (Figure 4A)[#].	A neuron is spatially manipulated to explain knowledge of its anatomy with kinesin, dynein and mitochondrion (Figure 4B).
	Visualize levels of organization	Relative size and scale of neuron cell body and axon depicted (Figure 4A).	Relative size and shapes of cell body, axon, motor proteins and mitochondrion depicted (Figure 4B).
	Decode a representation		Decode the symbolic language composing provided 'Neuron Assessment' figures (Figure 2a-c).
b. Organelle movement in neurons		Lack of evidence as no mitochondria or organelle movement represented (Figure 4A).	Lack of evidence as no organelle movement represented in visual representation of neurons (Figure 4B).
		RED areas	
c. Experimental design table RED areas: Control group[20] Treatment group[18]	**Interpret a representation**	Lack of evidence	'Neuron Assessment' figures were interpreted to design experimental groups (Figure 2a-c).
	Construct a representation		Experimental table constructed to represent manipulation of control and treatment variable groups (Figure 4C).
d. Graphs RED areas: Manipulation of variable[17] Measurement of outcome[7] Accounting for variability[22] Scope of inference[15]	**Construct a representation**		Constructedgraph is flawed as inappropriate independent variables are represented on x-axis) [2], [3]. Bars on the graph do not correspond to the experimental table and carry no error bars [4] (Figure 4D).
	Translate horizontally		Experimental table translated inappropriately into a graph as the

[0]Superscripts refer to the concepts listed in column 1 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php.

Supplement F Table 4:Juan's reasoning with visualizations (RM) before and with 'Neuron Assessment'			
	across a representation		experimental table groups do not correspond to the bars on the graph[5] (Figure 4D).

Correct ideas Difficulties Lack of evidence

Supplement F Table 5:Eve's reasoning with visualizations (RM) before and with 'Neuron Assessment'

Concepts	**RM***	**Before**	**With**
		Neuron subject matter	
a. Neuron knowledge	**Spatially manipulate a representation**	Manipulated figures of a neuron to explain knowledge of neuron anatomy (Figure 5A)#.	
	Visualize levels of organization	Depicted relative size of neuron cell body and axon (Figure 5A).	
	Decode a representation		Decoded the symbolic language composing provided 'Neuron Assessment' figures (Figure 2a-c).
	Translate horizontally across representations		Translated across provided representations of neuron and created own visuals of a neuron (Figure 5C).
b. Organelle movement in neurons	**Spatially manipulate a representation**	Spatial manipulation is flawed as mitochondrion is depicted in cell body but shows no movement (for example by using arrows) (Figure 5A).	Lack of evidence as no organelle movement represented in neuron figures (Figure 5C).
		RED Areas	
c. Experimental design table/figure	**Visualize levels of organization**	Relative size and scale of neurons depicted at the organ and cellular level	

*RM refers to Reasoning about the symbols and Modes of representations listed in Table 1

Control group[20] Treatment group[18]		(Figure 5B).	
	Interpret a representation		Provided 'Neuron Assessment' figures are used to design experimental groups (Figure 5D).
	Construct a representation		Experimental table represents control and treatment group variables[2] (Figure 5D).
d. Graphs RED areas: Manipulation of variables[17] Measurement of outcome[7] Accounting for variability[22] Scope of inference[15]	**Construct a representation**	Lack of Evidence as no graph was drawn (Figure 5B).	Graph drawn with independent variable on x-axis and dependent variable on y-axis [2, 3.] Different treatments are represented as separate bars (Figure 5E).
	Translate horizontally across representations		Experimental table translated graphically with treatments shown as separate bars on the graph appropriately[5] (Figure 5E).

Correct ideas Difficulties Lack of evidence

[θ] Superscripts refer to the concepts listed in column 1 and defined in Supplement A and online at http://www.scicard.net/glossary/index.php.

Supplement F Table 6: Li Na's reasoning with visualizations (RM) before and with 'Neuron Assessment'

Concepts	RM*	Before	With
		Neuron subject matter	
a. Neuron knowledge	**Spatially manipulate a representation**	Figure of drawn neuron was used to explain knowledge of neuron anatomy (Figure 6A)#.	Lack of evidence as no new representations were created to depict neurons.
	Visualize levels of organization	Relative size and shapes of cell body, axon and mitochondria depicted (Figure 6A).	
	Interpret the temporal resolution of a representation	Showed signal transmission as a mode of neuron communication (Figure 6A).	
b. Organelle movement in neurons		Lack of evidence as figures depict movement of signals but no movement of mitochondria (Figure 6A).	Decoded the symbolic language composing provided 'Neuron Assessment' figures (Figure 2a-c).
		RED Areas	
a. Experimental design table/figure Control group[20] Treatment group[18]	**Interpret a representation**	Lack of evidence	Provided neuron visuals were used to design experimental groups (Figure 6B).
	Construct a representation		Table constructed to depicted manipulated control and treatment variable groups (Figure 6B).
b. Graphs RED areas: Manipulation of variables[17] Measurement of outcome[7] Accounting for variability[22]	**Interpret a representation**		Provided 'Neuron Assessment' figures were used to design experimental groups (Figure 6C).
	Construct a representation		Graph constructed to represent control and treatment variable groups and independent variables and dependent variables were represented on x- and y-axes respectively) [2, 3] (Figure 6C).

Scope of inference[15]	**Translate horizontally across a representation**		Experimental table was translated into a graph representing each treatment in the table as separate bars appropriately (Figure 6C) [5].

Correct idea Difficulties Lack of evidence

Supplement F Table 7: Daniel's reasoning with visualizations (RM) before and with 'Neuron Assessment'

Concepts	RM*	Before	With
		Neuron subject matter	
a. Neuron knowledge	**Spatially manipulate a representation**	Figure of a neuron manipulated to explain knowledge of neuron anatomy (Figure 7A)#. Relative size and shapes of axon and dendrites depicted.	Lack of evidence as no new representations were created to depict neurons.
	Visualize levels of organization		
b. Organelle movement in neurons		Lack of Evidence as no depiction of organelle movement (Figure 7A).	Decoded the symbolic language composing provided 'Neuron Assessment' figures (Figure 2a-c).
		RED areas	
c. Experimental design table/figure Control group[20] Treatment group[18]	**Interpret a representation**		Provided neuron visuals were used to design experimental groups (Figure 7C).
	Construct a representation	Experimental groups[2] considered and measurement of outcome[3] (Figure 7B).	To represent manipulation of control and treatment variables groups[2] (Figure 7C).
d. Graphs Manipulation of variables[17] Measurement of outcome[7]	**Construct a representation**	Lack of Evidence as no graph was drawn (Figure 7B).	Graph constructed with independent variables and dependent variables[2, 3] on x- and y-axes respectively. Different treatment groups are represented as separate bars (Figure 7D).
	Translate horizontally across representations		Experimental table translated into graph representing each treatment in the table as separate bars appropriately (Figure 7D).

Correct idea Difficulties Lack of evidence

Supplement F Table 8: Juan's abilities with reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
			Neuron subject matter		
(I) Neuron concepts	a. Neuron knowledge	*"Neuron has an axon. And mitochondria".*	Memorize parts of neuron anatomy.	*"I am familiar with how a neuron looks with axons."*	Memorize parts of neuron anatomy.
	b. Organelle movement		Lack of evidence	*"Scientists want to see if kinesin or dynein malfunction is responsible in causing the disorder. Anterograde and retrograde movement in neurons takes place with help of kinesin and dynein".*	Apply knowledge of neurons, molecules like kinesin, dynein and mechanisms like antero- and retrograde movement to explain investigation goal of diagnosing impaired mitochondrial movement mechanism.
			RED areas		
(1) Variable property of experimental subject	a. Experimental subject *Sample*[13#] *Subject*[16] *Unit*[19] *Variable*[20]	*"[Scientists] would do individual experiments on mitochondria, kinesin and dynein*[16]*. They could remove kinesin and see that the mitochondria will only move*[20] *one way."*	Integrate knowledge of neuron concepts (mitochondria, kinesin, and dynein) [16] with the experimental subject and its variable property (movement of mitochondria) [20].	*"Neurons* [16, 19] *that lack kinesin* [20] *and neurons that lack dynein".* (RED, Area of Difficulty 1-b)	Apply knowledge of neuron concepts (kinesin and dynein) to propose a variable property of the experimental subject[16]. The variable property (neurons lacking kinesin) [20] is not aligned to the investigation goal.
(2)	a. Treatment	*"[Scientists] could*	Integrate knowledge	*"Use compound K* [17]	Reason globally about

Supplement F Table 8: Juan's abilities with reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
Manipulation of variables	variable *Subject*[16] *Treatment variable*[17] *Treatment group*[18]	*remove kinesin*[17, 18] *and see that the mitochondria*[16] *will only move one way."*	of experimental subject[16] (kinesin, mitochondria) to propose treatment variables[17] (removal of kinesin).	*on neurons that lack kinesin* [18] *and compound D*[17] *on neurons that lack dynein*[18]*".* (RED, Area of Difficulty 2-d)	treatment variables[17] (treatment with compound K to neurons lacking kinesin[18] confounds the experimental goal of investigating the disorder).
	b. Control variable *Control*[1] *Control group*[2]		Lack of evidence	*"They will select a patient with a disorder as control and one without the disorder and compare* [2]*".* (RED, Area of Difficulty 2-j)	Transfer and apply knowledge of concept of control groups[2] for comparison purposes.
	c. Controlling outside variables *Confounding variables*[8#] *Control group*[2] *Treatment group*[18]		Lack of evidence		Lack of evidence
(3) Measurement of outcome	a. Outcome *Variable*[20] *Subject*[16] *Outcome variable*[7]	*"[Scientists] would be measuring the degree of necessity of a certain motor protein*[7, 20]*".* (RED, Area of Difficulty 3-e)	Apply knowledge of the concept outcome variable[7, 20] to propose a suitable measure.	*"They would be measuring movement*[7] *of mitochondria to see if it changes without the protein".* (RED, Area of Difficulty 3-	Apply knowledge of the concept outcome variable[7] to propose a suitable measure. *No specific outcome proposed here (measurement of change in movement is not specific indication of a*

Supplement F Table 8: Juan's abilities with reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
				e)	*measure).*
(4) Accounting for variability	a. Replication *Variability*[22] *Subjects* [16] *Units* [19] *Treatment group* [18] *Control group*[2]		Lack of evidence		Lack of evidence
	b. Randomization *Randomization*[1] [1] *Random sample* [10] *Treatment groups* [18] *Variability* [22]				
	c. Representative sample *Random sample* [10] *Control group* [2] *Treatment group*[18]				

Supplement F Table 8: Juan's abilities with reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
(5) Scope of inference	a. Scope of Inference[15#]	*"If [scientists] find a problem with kinesin and/or dynein, they could manufacture genetically some substitute for the missing motor proteins and observe the effect[15]".* (RED, Area of Difficulty 5-b; 5)	Reason locally (replacing genetically modified kinesin with impaired kinesin) and globally to make appropriate inferences[15] from experimental findings (scope of inference for patients with a neuronal disorder).		Lack of Evidence
	b. Cause and effect *Treatment Variable[17] Outcome variable Confounding Variables [8] Correlations[3]*		Lack of Evidence	*"When kinesin is lacking and thus, replaced with a genetically modified version of kinesin protein[17], the patient showed improvement in mitochondrial movement[7, 3,8]".* (RED, Area of Difficulty 5-c)	Apply knowledge of treatment[17], control[1]and outcome[7] variables to develop causal [3.4] explanations (causal explanations are made with respect to a mismatched treatment variable and no variability measures are considered)

Correct ideas Difficultie Lack of evidence

Supplement F Table 9: Eve's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
		Neuron subject matter			
(I) Neuron concepts	a. Neuron knowledge	*A neuron is connected to other axons to distribute information*	Memorize knowledge of neurons and axons.	*In psychology I have seen similar types of neurons and axons in the brain.*	Apply knowledge of neurons to interpret the experimental context.
	b. Organelle movement	*What's going on in the mitochondria determines how [organelle] transport occurs".* (RED, Area of Difficulty 1-b)	Reason locally (mitochondrial process) and globally (processes inside mitochondria regulate organelle movement).	*People with the disorder are unable to perform transport and scientists believe that it has to do with motor proteins-kinesin and dynein not working and it effect on movement of mitochondria.*	Apply knowledge of concepts like transport, kinesin, dynein, mitochondria to explain the investigation goal.
		RED Areas			
(1) Variable property of experimental subject	a. Experimental subject *Sample*[13#] *Subject*[16] *Unit*[19] *Variable*[20]	*"[Scientists] would have to take a living specimen of the neurons*[13, 16] *and keep it in the environment to function properly and observe how it affects overall transport*[20]*."*	Apply knowledge of the neuron[16] concepts (living cells) to propose experimental subjects and its variable property[20] (transport).	*"You can try a neuron with only kinesin* [16, 20] *and inject compound K".* (RED, Area of Difficulty 1-b)	Apply knowledge of experimental subject [16] but the variable[20] property is not aligned with the investigation goal (impaired neurons with only kinesin with not allow unbiased investigation of

Supplement F Table 9: Eve's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
					whether kinesin and/or dynein are the source of the neuron disorder).
(2) Manipulation of variables	a. Treatment variable[17] *Treatment group*[18]	*"[Scientists] would inject*[17] *what they need, to manipulate things in the processes of the neurons."*	. Apply knowledge of the treatment variable[17] (injection of compounds)	*"Add compound K*[17] *to neurons with only kinesin*[18]*; compound D to neurons with only dynein".* (RED, Area of Difficulty 2-d)	Reason locally (inject compound K to neurons only carrying kinesin) and globally (using neurons with only kinesin confounds the experimental goal of investigating whether kinesin or dynein are responsible for the neuron disorder) about treatment variables [17]
	b. Control variable *Control*[1#] *Control group*[2]	*"[Scientists] are going to need the control*[1, 2] *which would be people that don't have the disorder so healthy neurons and experiment would be people that carry the unhealthy neurons."* (RED, Area of Difficulty 2-j)	Reason globally about control[1, 2] (Experimental subjects carrying obvious differences are assigned to experimental vs. control group.)	*"Neurons without any proteins*[2] *[kinesin or dynein]*". (RED, Area of Difficulty 2-h)	Transfer and apply knowledge of control (control group[2] does not provide natural behavior conditions because absence of the manipulated variable in treatment group,

Supplement F Table 9: Eve's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
					results in conditions unsuitable for the experimental subject.)
	c. Controlling outside variables *Confounding variables*[8] *Control group*[2] *Treatment group*[18]		Lack of evidence	*"Neurons in control*[2] *and experimental group*[18] *with both carry same organelles*[8]"	Apply knowledge of controlling confounding variables[8] to have uniform experimental subjects in control[2] and treatment[18] groups.
(3) Measurement of outcome	a. Outcome *Variable*[20] *Subject*[16] *Outcome variable*[7]	*"[Scientists] would observe to see what happens if they specifically change a certain thing*[7]*".* (RED, Area of Difficulty 3-f)	Apply knowledge of outcome variable[7] to propose a suitable measure.	*"Measure mitochondrial*[16] *movement*[7, 20] *[after treatment with compound K and D each] and compare with healthy amount of movement*[7]*".* (RED, Area of Difficulty 3-e)	Apply knowledge of outcome variable[7] to propose a suitable measure. *No specific outcome proposed here (healthy amount of movement is not specific indication of a measure).*
(4) Accounting for variability	a. Replication[12] *Variability*[22] *Subjects* [16] *Units* [19] *Treatment group*[18]	*"[Scientists] have to get a significant amount of samples to test. But you need to do the experiment multiple times and so you would have*	Apply knowledge of replication[12] to propose multiple trials of the experiment but at		Lack of evidence

Supplement F Table 9: Eve's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
	Control group[2]	*to have a decent amount of neurons*[16] *from the healthy and unhealthy patients to conduct the experiment to compare if results are significantly close to each other*[22]*, otherwise the experiment really wouldn't be accurate. Multiple trials must be done."*	another time as measure of dealing with variability[22].		
	b. Randomization[11#] *Random sample* [10] *Treatment groups*[18] *Variability* [22]		Lack of evidence		Lack of evidence
	c. Representative sample *Random sample* [10] *Control group* [2] *Treatment group*[18]		Lack of evidence	*"The control will be the healthy neuron*[2] *but experimental group will be the unhealthy neurons*[10, 18]*"*. (RED, Area of Difficulty 1-b)	Apply knowledge of representative (random) sample [10] to treatment[18] and control[2] group subjects.
(5) Scope of inference	a. Scope of inference[15]		Lack of evidence	*"When you see movement with kinesin and dynein inhibitor is equal to the control movement of healthy cell, your experiment is successful"*[15]. (RED, Area of Difficulty 5-c)	Reason locally (presence of inhibitors) and globally (treatment with kinesin/dynein inhibitors will result in healthy neuron

Supplement F Table 9: Eve's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
					movements) about experimental inferences[15] don't align with provided background.
	b. Cause and effect[4] *Treatment Variable* [17] *Outcome Variable* [7] *Correlations*[3]	*"[Scientists] inject what they need to* [17] *manipulate things to see what happens if they specifically change a certain thing- and how it affects the overall transport*[7] *".* (RED, Area of Difficulty 5-c)	Reason globally about causal claims (a causal relationship is claimed even though the data only show correlational[3] association between variables.)	*"With the [presence of] proteins individually, there might be loss in mitochondrial movement. But with both inhibitors* [17]*, that is going to have full movement close to the control"*[3, 4, 7,8]*.* (RED, Area of Difficulty 5-c)	Reason locally (presence of inhibitors) and globally (presence of inhibitors will result in healthy neuron movements) about causal relationship between treatment[17] and outcome variables[7] that do not align with provided background.

Correct idea Difficu es Lack of evidence

Supplement F Table 10: Li Na's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
		Neuron subject matter			
(I) Neuron concepts	a. Neuron knowledge	*"Neurons transfer signals […] the neuron can transmit that information to your brain"*	Memorize knowledge of 'signal transmission' and 'neurons'	*"Neurons have different terminals like cell terminal and there is a cell body"*	Memorize knowledge of 'neuron anatomy'
	b. Organelle movement	*"Neurons communicate with each other and gradual change in ions across a membrane help in transmitting signals along axons"* (RED, Area of Difficulty 1-b; 1-c)	Apply knowledge of neuron concepts to explain organelle movement	*"Mitochondria are along the axon of a neuron. Kinesin and dynein can cause movement in different directions of mitochondria"*	Apply knowledge of neuron concepts to explain organelle movement
		RED Areas			
(1) Variable property of experimental subject	a. Experimental Subject *Sample*[13#] *Subject*[16] *Unit*[19] *Variable*[20]	*"[Scientists] would amplify the process [in the neuron]* [16] *and label some important organelles*[20]*"*	Integrate knowledge of neuron[16] knowledge (neuron, organelles) to propose experimental subject and its variable property[20]	*"The sample/subject* [13, 16] *is the mitochondria in the neuron and kinesin/dynein is the variable which will be either inhibited or*	Apply knowledge of the neuron[16] (mitochondria, neurons, kinesin/dynein) to propose an experimental subject with variable

Supplement F Table 10: Li Na's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
			(amplification of neuronal process and labeling organelles)	*activated*[20] *"*	property[20] (activation/inhibition of kinesin/dynein)
(2) Manipulation of variables	a. Treatment variable *Subject*[16] *Variable*[20] *Treatment variable*[17] *Treatment group*[18]	*"[Scientists] might have labeled*[17] *the important organelles* [16] *"*	Transfer and apply the knowledge of treatment variables[17] applied to a treatment group [18] of experimental subjects [16]	*"Experimental groups will be: activate kinesin*[20] *and inhibit dynein/ activate kinesin and dynein/ inhibit kinesin and activate dynein* [17, 18] *"*	Apply knowledge of treatment variable[17] (kinesin and dynein inhibitors) to propose suitable treatments (activation/inhibition) applied to experimental subjects [16]
	b. Control variable *Control*[1#] *Control group*[2]		Lack of evidence	*"Neurons treated with kinesin and dynein inhibitors will be the control group* [1, 2] *"*. (RED, Area of Difficulty 2-i)	Reason globally about control group[2] (control group needs to carry neurons in natural condition as inhibition of organelle movement in neurons will not allow comparison to treatment groups).
	c. Controlling outside variables *Confounding variables*[8] *Control group*[2] *Treatment group*[18] *Variation*[21]		Lack of evidence	*"Before the treatments subjects should have the same conditions* [8, 21] *in the treatment and control groups* [2, 18]*. Otherwise, they may react differently leading*	Apply knowledge of the controlling outside variables8 (experimental subjects subjected to same conditions) in treatment[18] and control groups[2] as a measure to reduce

Supplement F Table 10: Li Na's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
				to false causation"	variation[21]
(3) Measurement of outcome	a. Outcome *Variable*[20] *Subject*[16] *Outcome variable*[7]	*"[Scientists will] measure which organelle will cause movement in different directions*[7]*; They could measure the direction and displacement or electrical potential*[7, 20]*,,*	Apply knowledge of a specific measureable outcome[7] that the experimental subject[16]carries in response to experimental conditions (The outcome proposed here is not in response to experimental but natural conditions).	*"Displacement of mitochondria*[7, 16, 20] *can be measured in the form of length in micrometers"*	Apply knowledge of a specific measureable outcome[7] that the experimental subject[16]carries in response to experimental conditions
(4) Accounting for variability	a. Replication[12] *Variability*[22] *Subjects* [16] *Units* [19] *Treatment group* [18] *Control group*[2]		Lack of evidence	*"We need to use a large number of samples*[16] *in treatment* [18] *and control groups* [2]*, to observe data outliers*[22] *and then just decide values that lie centrally"*	Apply knowledge of replication[12] to experimental subjects[16] (large number of samples) as measure to reduce variability[22]
	b. Randomization[11] *Random sample* [10] *Treatment groups*[18] *Variability* [22#]		Lack of evidence	*"Neurons need to be picked at random and assigned to treatments completely randomly*[11, 22]*. You*	Apply knowledge of random sampling[10] and randomization[11] (random assignment of treatments in treatment groups[18]) as measure to reduce

Supplement F Table 10: Li Na's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
				consider that all cells are the same and randomly assign[11] *them to the experimental groups"*	variability[22]
	c. Representative sample[10] *Sample* [13] *Random sample* [10] *Control group* [2] *Treatment group*[18]		Lack of evidence	*"[For both treatment*[18] *and control groups*[2]*] I will keep the same organelles under observation*[13]*, use the same species of organisms for the neurons and use cells from the same one animal. And also make sure that they are in the same environment"*	Apply knowledge of selecting a representative random sample[10, 13] in the treatment[18] or control[2] group (organism species and cells) as a measure to average out variations
(5) Scope of inference	a. Scope of inference[15]				Lack of evidence
	b. Cause and effect[4] *Treatment Variable* [17] *Outcome variable* [7] *Correlations*[3]		Lack of evidence	*When kinesin is activated and dynein is inhibited*[17]*, we see movement in the anterograde direction*[7]*. When dynein is working and kinesin is*	Reason globally about causal claims (contradictory correlation[3] relationship between treatment[17] and outcome[7] variables is suggested)

Supplement F Table 10: Li Na's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

Concepts	Before (Phase 1)	RC	With (Phase 2)	RC
			inhibited[17] *we see movement in the retrograde direction*[7]*. When both are activated, the functions of the two proteins are replicated and thus, the mitochondria cannot move in either direction so the movement is impaired* [3]*."* (RED, Area of Difficulty 5-c)	

Correct idea Difficulties Lack of Evidence

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
			Neuron subject matter		
(I) Neuron concepts	a. Neuron knowledge	i. *"Nerves carry signals throughout your body to move or other processes".*	Memorize entities: nerves and signal transmission processes	ii. *"Neurons have axons and a branched structure".*	Memorize entities: axon structure
	b. Organelle movement	i. *"I just think of electrical signals that would move against the wall of the neuron".* (RED, Area of Difficulty 1-b)	Apply knowledge of neuron concepts (signal transmission) to explain organelle movement	ii. *"Two proteins help in the movement. One protein goes one way and the other goes the other way. They move along an axon of a neuron."*	Integrate knowledge of structure and function of neuron concepts (two proteins, axon) to explain organelle movement mechanism
			RED Areas		
(1) Variable property of experimental subject	a. Experimental subject[#] *Sample*[13] *Subject*[16] *Unit*[19] *Variable*[20]	i. *"An experiment involving people*[16] *with impaired nerves*[20]*".*	Apply knowledge of variable[20] property (impairment of nerves) to experimental subject[16].	ii. *"There are two different compounds to inhibit two different proteins and observe which inhibited protein affects mitochondrial movement in neurons* [16,20]*".*	Apply knowledge of experimental subject[16] (neurons) and variable property[20] (mitochondrial movement under the effect of

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
					proteins)
(2) Manipulation of variables	a. Treatment variable *Subject*[16] *Variable*[20] *Treatment variable*[17] *Treatment group*[18]	i. *"[Scientists] would compare signals*[20] *among people in the control groups with the experimental group*[18] *that have an impaired nervous system*[17, 20]*"*.	Apply knowledge of treatment group[18] of experimental subjects[16] exposed to experimental conditions that vary[20] (varying signals in control vs. experimental groups) in a certain way.	ii. *"Split cells of normal persons into 5 different groups*[18]*. Each group carries a different treatment [normal person; control with no treatment, one with compound K*[20] *and another one with compound D*[20]*; one gets both]"*	Apply knowledge of treatment group[18] of experimental subjects[16] exposed to experimental conditions that vary[20] (varying compound treatments) in a certain way.
	b. Control variable *Control*[1] *Control group*[2]	i. *"Comparing with a control group with people that have normal/regular nervous system*[1, 2]*"*. (RED, Area of Difficulty 2-j)	Transfer and apply the knowledge of the concept of control[1, 2]. Reason globally about the concept of control[1, 2] (Experimental subjects carrying obvious differences are assigned to experimental vs. control group).	ii. *"The control group*[1, 2] *would not be receiving any treatment but would still be subjected to the same conditions as the treatment group"*.	Reason globally about the concept of control[1,2] (Parameters other than the treatment variable are identical for both treatment and control conditions).
	c. Controlling outside variables *Confounding variables*[8#]	i. *"[Scientists] would try to keep people as similar*[8,21] *as*	Apply knowledge of controlling outside variables[8, 21] by matching control[2] and	ii. *"People (in treatment*[18] *and control*[2] *groups) need as similar as possible, in health conditions, so that we know that the*	Apply knowledge of controlling outside

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
	Control group[2] *Treatment group*[18] *Variation*[21]	*possible so it's just the nervous system that's different between the two (treatment*[18] *and control*[2]*) groups so results aren't affected".*	treatment[18] groups as closely as possible.	*observed effect is due to compound K or D application*[18]*".*	variables[8, 21] by matching control[2] and treatment[18] groups as closely as possible to draw clear causal claims.
(3) Measurement of outcome	a. Outcome *Variable*[20] *Subject*[16] *Outcome variable*[7]	i. *"You could measure the strength of the electrical signals or the path the signal takes*[7, 20]*".* (RED, Area of Difficulty 3-c)	Apply knowledge of outcome variable[7] to propose a suitable measure (association of measuring strength of electrical signals with measurement of organelle movement is not explained).	ii. *"I predict with treatment of compound K, the mitochondria moved 4 units less than the control groups it over a specific period of time*[7,20]*".*	Apply knowledge of outcome variable to propose measureable outcomes.
(4) Accounting for variability	a. Replication[12] *Variability*[22] *Subjects* [16] *Units* [19] *Treatment group* [18] *Control group*[2]	*i. "Scientists would try to measure the electrical signals in the two different groups* [2, 12,16,18,19].	Apply knowledge of replicating[12] measurements in groups of experimental subjects[16] across treatment[18] and control groups[2] as a measure to reduce variability[22].	ii. *"I would use groups*[12] *of neurons*[16] *for each experimental group*[2, 18]*".*	Apply knowledge of replicating[12] measurements in groups of experimental subjects (neurons) [16] in each experimental

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

	Concepts	**Before (Phase 1)**	**RC**	**With (Phase 2)**	**RC**
					group[2, 18] as a measure to reduce variability[22].
	b. Randomization[11#] *Random sample [10] Treatment groups [18] Variability [22]*	*Lack of Evidence*		ii. *"I would randomly assign cells[11] into groups[18] to avoid biasing[22] the results and only measure effect of the compounds".*	Apply knowledge of 'randomization'[11] of treatment group[18] conditions as a measure to reduce variability[22] and bias in the experiment.
	c. Representative Sample *Sample [13], Random sample [10], control group [2], treatment group[18]*	*Lack of Evidence*		ii. *"Use a sample of patients with the same age range, height etc[10, 13] so that only the neurons are different between the two groups[2, 18] to avoid biasing the results".*	Apply knowledge of 'representative sample' [10, 13] selection in treatment[18] and contro[l2] groups as a measure to reduce bias experimental results.
(5) Scope of inference	a. Scope of Inference *Scope of Inference[15]*	i. *"If there is a difference between heights of*	Reason globally about inference[15] of experimental results (difference in electrical	ii. *"Compare the movement with multiple patients who have the disorder with the 4 groups of patient. This will allow us to*	Reason locally and globally (variability measures,

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

Concepts	Before (Phase 1)	RC	With (Phase 2)	RC
	subjects in two different groups, you wouldn't be able to necessarily decide if it was the height that gave rise to the difference in strength of the electrical signals rather than the nervous system[15]". (RED, Area of Difficulty 5-b)	signal strength is an irrelevant variable and thus inferences are made to an irrelevant target population).	*infer that those were the protein that caused the disorder[15]".*	suitable control and experimental groups, movement as the variable property and measureable outcome variable) to draw inferences[15] about the protein impairment leading to the neuronal disorder.
b. Cause and effect[4] *Treatment Variable [17], control[1] and outcome variable[7], Correlations[3]*	i. *"You could measure the strength of the electrical signals[7] or the path the signal takes and see differences in sending signals [3,7]".* (RED, Area of Difficulty 5-b)	Integrate knowledge of relevant measurable outcome variables[7] to draw appropriate causal explanations. Reason globally to claim a causal relationship[4#] separate from correlations[3] (measurement of electrical signals is	ii. *"Compare your treatment groups"[1, 17] movements with movement in neurons of a patient with disorder to see similarities in trends of the movement. If they did have the same movement[7], you could argue the source of the disorder as per your treatment [3, 4]".*	Reason locally (comparison of trends in mitochondrial movement in neurons) and globally (comparison of movement trends, along with variability measures lead to

Supplement F Table 11: Daniel's abilities reasoning with concepts (RC) before and with 'Neuron Assessment'

Concepts	Before (Phase 1)	RC	With (Phase 2)	RC
		mismatched with investigation goal).		the protein source that leads to the neuron disorder) about the causal relationship[4] as separate from correlations[3] between treatment[17] and outcome variables[7].

Correct ideas Difficulties Lack of Evidence