

The Axonal Highway

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Abstract:

The complexity of how the carrier proteins work, how the cargo is attached to the proteins, and how different diseases affect axonal transport are all the subject of intense research. Through a brief understanding of this research, my partner and I developed an axonal transport model. The purpose of this model was to teach axonal transport to fifth graders by instructing them in the principles of intracellular transportation using microtubules, protein transporters, and vesicles. We did this by using a display board to familiarize the children with the process of axonal transportation and then letting the children perform axonal transportation in a large model neuron all while having fun by competing against their fellow classmates. From the results of the evaluations, it was clear that the fifth graders not only had fun in the relay race, but comprehended how axonal transportation works.

Introduction:

A large component of axonal transportation is transportation of mitochondria along microtubules. MitoTracker dyes and confocal microscopy helped to reveal properties of mitochondrial transport in guinea-pig myenteric neurites. Significant results were discovered in three areas of axonal transport: mitochondrial staining in myenteric neurons, transport of mitochondria in nerve fibers, properties of mitochondrial transport. The mitochondria were stained using a specific dying and fluorescing process that involved MitoTracker Green being injected into the mitochondria so they could be monitored as they moved down the axon. The mitochondria were found to be transported bidirectionally having both antero- and retrograde movement. This research revealed that mitochondria don't travel in the same pattern down the axon. Furthermore, mitochondria occasionally reversed directions in the middle of a tract without influencing other mitochondrial paths (Vanden Berghe).

Some properties of transportation, such as stationary periods, followed by highly active periods of movement were observed, showing that the cell does not maintain a constant level of activity. There are times when more mitochondria are needed at the terminal or in the soma, and times when less are needed. This is an important property of the neurons transportation system. The load and length of the mitochondria's travel also have an effect on the overall velocity that transportation. Different velocities reinforce the idea of dissimilar transportation. The researchers showed that when mitochondria are transported they change velocity periodically. Other conclusions included: transport is independent of track specificity, and stationary mitochondria do not effect the overall transportation on mitochondria. Furthermore, this study gave means by which other researchers could perform further research on axonal transportation (Vanden Berghe).

Further research has been done in the area of specialization of motor proteins to carry particular cargo to precise locations. It was shown that during *in vivo* transport proteins move bidirectionally with only the overall movement in the retro or anterograde direction. The motor proteins pause and reverse direction throughout their travel on the axon. Analysis of transport parameters through experiments with Dynactin a multiprotein complex involved in dynein mediated transportation revealed the patterns of bidirectional movement (Kamal A). Dynein inhibited anterograde transport from disruption of dynein and showed competitive interaction with opposing motor proteins. More research is needed on the topic of bidirectional movement of motor proteins to understand the interaction of kinesin and dynein (Brian W Guzik).

Another key aspect of axonal transport is the binding of the cargo to the protein transporter. It was demonstrated that KIF1A, monomeric Kinesin, binds to liposomes by the PH, pleckstrin, by interactions with (PtdIns(4,5)P₂) (Lemmon MA). This interaction was not found to induce transport but a large sensitivity to changes of PtdIns(4,5)P₂ concentrations were observed suggesting that upstream signals might regulate the transport of Kinesin's cargo (Brian W Guzik).

Neurogenetic diseases are caused by errors within the transport system. Axonal clogging has been linked to Huntington's disease through disruption of the Htt gene in *Drosophila*. Poly Q proteins (polyglutamine) inhibited motor components of axonal transport (Szebenyi G). Poly Q was also shown to decrease transportation bilaterally when applied directly to a giant squid axon. Through damage to the transport system, researchers are better able to understand the normal function of the system (Brian W Guzik).

Research has also shown diseases such as hereditary spastic paraplegia (HSP) involving axonal degradation that occur before neuronal death. A model of HSP was developed in the mouse which is 88% identical genetically to people with HSP. These mice lack paraplegin which leads to abnormal mitochondria in synaptic terminals and distal regions of long axons, problems with axonal transport and axonal degeneration (Ferreirinha F).

Discrepancies in retrograde transportation were also found to greatly affect axonal degradation. This might occur by affecting the transport of mitochondria, the trafficking of endosomes, and the internalization of neurotrophic factors. Loss of mitochondrial function may affect axonal transport and cause axonal degeneration, which if reversed, may prevent the loss of axons and help people with HSP (Ferreirinha F).

Disorders involving the axonal transport system have allowed researchers to understand and test the different ways in which transportation of machinery occurs. The better we understand the pathways of neurological diseases the closer we are to fixing them or providing different pathways (Brian W Guzik).

Our axonal highway model was based upon anterograde and retrograde axonal transport that takes place in neurons. Neurons contain microtubules upon which proteins travel the length of the axon. The proteins used include: kinesin, which is specific for anterograde transport, and dynein for retrograde transport. These proteins carry synaptic vesicles from the cell body to the presynaptic terminal or from the terminal back to the axon. The vesicles contain neurotransmitters that act on postsynaptic cells. Specifically we wanted the kids to learn concepts of axonal transportation.

Methods:

In order to convey concepts of axonal transportation, my partner and I used a display board and model neuron. We utilized the display board to explain the principles of axonal transport to the children; including why the body needs it.

The model neuron had many different components which represented different parts of a neuron and the proteins involved in axonal transportation. We set up the model neuron using duct tape to outline the cell body, dendrites, axon, and axon terminal. Dimensionally, the neuron was approximately forty feet by ten feet which allowed the children to stand and move within it. Microtubules, illustrated by two parallel ropes, ran from the soma to the axon terminal. Mitochondria were symbolized by buckets of candy placed in both the soma and the presynaptic terminal. The candies in the mitochondria represented the ATP utilized by the cell to energize the mechanism of transport. The children themselves corresponded to transport proteins: either kinesin or dynein. They carried synaptic vesicles containing neurotransmitters simulated by plastic egg shells holding several candies (Figure 1).

The children split into two groups; a retrograde group and an anterograde group. They received colored labels to distinguish whether they were dynein or kinesin. Then the kids took a piece of candy from the mitochondria which gave them the energy required to perform the task. Next, they grabbed a synaptic vesicle, either from the soma or presynaptic terminal depending upon which protein they were assigned, and traveled hand over hand backwards to the opposite end of the axon. When they reached their destination, they released the neurotransmitters from the synaptic vesicle. The children raced each other in groups, like a relay race, in opposite directions to add an element of fun. At the end of the activity we quizzed the children to determine whether they understand the fundamental concepts of axonal transport (Figure 2).

Figure 1

Figure 2

The materials used included a lot of room, ropes, hollow balls, small objects, candy jar, candy, display board, two large tubs, and colored labels.

Results:

After the kids listened to our presentation and completed the relay race they evaluated our project by giving us ratings 1-5 on different questions, one being the worst and five the best. Then they were asked to write in answers to a few other questions. The results are as follows:

- 1. Could you understand what the presenters were trying to tell you?** Average: 4.38
- 2. Were presenters friendly?** Average: 4.95
- 3. Was the exhibit fun?** Average: 4.90
- 4. Would you like to learn more?** Average: 4.15

Summarized written entry:

- 1. What was your favorite part?** candy, relay game, learning
- 2. What did you learn?** vocabulary words, about the axonal highway, lots of things, not much, mitochondria, microtubules,
- 3. Additional comments:** it was great, I had a blast, the game was fun, great job, Chelsey needs to be more excited, I learned a lot.

The kids loved the model. They were all very enthusiastic and excited about competing against each other in the relay race. A few of the kids asked good questions. For example, one fifth grader asked why neurotransmitters couldn't just be released from the cell body. Another kid inquired whether the two types of proteins, kinesin and dynein, moved in different ways or if they were exactly alike. To give the kids an incentive for paying attention to how axonal transportation worked, we incorporated a question-answer session into the relay race. After the kids retrieved a synaptic vesicle from the opposite side of the neuron, they had to release the neurotransmitters before the next person could go. Before they were able to release the neurotransmitters they had to answer a question pertaining to a component of the axonal highway. These questions included, what is inside of the synaptic vesicle, what is the rope portion of the neuron that you were traveling on called, where did you get your energy from, are you involved in anterograde or retrograde transportation, and many more. If the fifth graders answered the question correctly then they could release the neurotransmitters immediately however, if they answered it incorrectly then we told them the right answer and asked another question until they answered correctly.

My partner and I received first place in our group. It was quite an honor to be picked by so many of the kids as the favorite exhibit. Although, I think part of the reason we received first place was due to the fact that we gave them candy and let them run around.

Discussion:

The children received the model well. The average of our scores per question was always in between four and five and we never received a score lower than 3. By this data it is clear that the kids not only enjoyed the exhibit but they also learned something from it. There was only one negative comment in the written entry portion of the critique. One kid said that he didn't learn much. This is to be expected, but over all 38/39 said that they had fun learning and enjoyed playing axonal highway game. By the results, it was a little hard to tell what the kids learned because most of them did not go into much detail when asked what you learned. Also, our display board allowed the kids to look at it and copy words directly from it if they desired.

The model was an excellent tool in showing kids the different components of axonal transportation. They were able to relate the vocabulary words and function of many different types of cell machinery to the model neuron through the relay game. The relay game made the kids think about what they had to do, as a transport protein, to get machinery to the other end of the neuron. The model did have limitations. In a real neuron, vesicles containing neurotransmitters have to go through a process of reuptake; but in our model neuron, the vesicles were prestocked. The processes

(anterograde transport and retrograde transport) were presented as the same (to make the game a fair race) when they are actually different.

To make the model more interesting, or more applicable to the scientific research being done in this field, it would be fun to have the kids try to complete the research with some obstacle in the way possibly representing Hutchinson's disease. The model tried to explain the process of specialization of motor proteins to carry particular cargo to precise locations (Brian W Guzik).

When we started presenting the model to the kids we ended up having time we needed to fill. We decided that we would have the kids eat the candy completely before they went on the microtubules and also they had to transverse the microtubules, travel numerous times from one end to the other end of the axon, before picking up a synaptic vesicle. Traveling back and forth many times before picking up cargo is not how dynein or kinesin work. This was a large limitation in our model. To fix this problem in the future, we could have the kids move by somersaulting or doing the crab walk backwards, anything that would take a long time to do. Another inconsistency in our model was that the neuron transmitters were released both in the axon terminal and in the soma. To resolve this problem in the future, we could have the kids carry empty synaptic vesicles back to the soma and have the kids fill them with neurotransmitters before handing them off to their partner. For the kids taking the synaptic vesicles from the soma to the terminal we could have them release the NT outside the cell.

This project was a wonderful experience. It taught us how to teach and present a hard topic in neuroscience to young children. We did well and had fun doing it.

References:

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