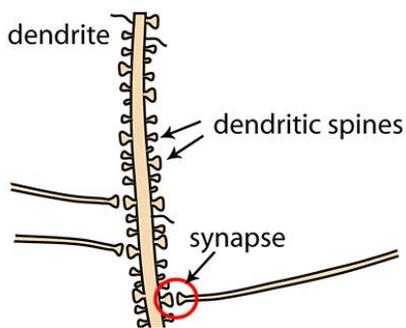


## Spine Lab Manual: NEU125

**Background: To read before class on 11/5**

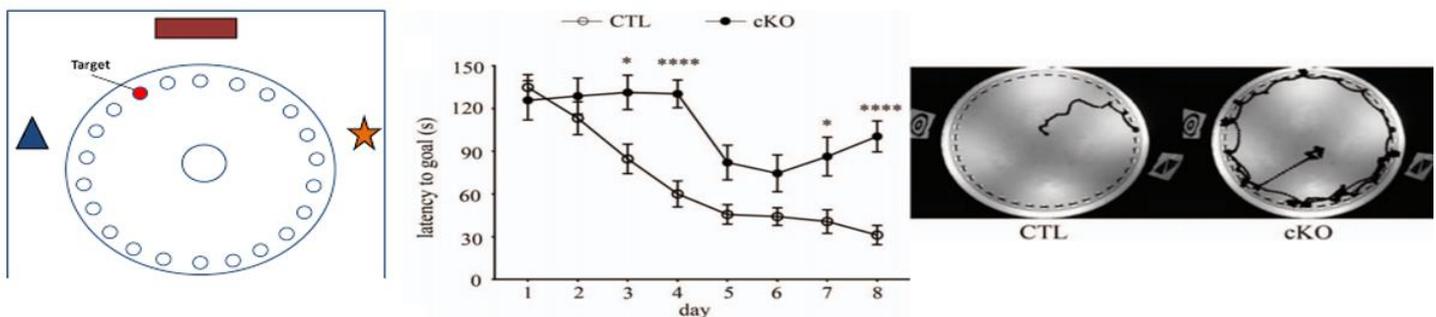
### Introduction

Intellectual disability (ID) affects more than 1% of the United States population, marking it as the most common developmental disorder. Individuals with ID are defined as having an IQ of less than 70 (100 is normal). Mechanisms underlying the development of ID are various, ranging from genetic, gestational insult, nutritional deficiencies in infancy and childhood, and trauma. CTNNB1 Syndrome is a form of ID in which the gene, *CTNNB1*, is mutated or partially deleted in such a way that it causes the protein it codes for,  $\beta$ -catenin (beta kuh-teen-in), to function less than typical. Over 200 individuals world-wide have *CTNNB1*, and ongoing research is centered around identifying (1) how losing function in  $\beta$ -catenin causes *CTNNB1* Syndrome and (2) what are the potential targets for pharmacotherapies.



One common theory of the development of ID is that the brain has trouble forming the appropriate connections between neurons (left). Synaptic spines are highly plastic (changeable) post-synaptic structures that change in shape as the synapse strengthens or weakens. The strengthening and weakening of synapses are the way in which the brain remodels itself to create new memories and learn new skills. Thus, if synaptic spines are not able to become weaker or stronger, then learning may not occur, suggesting a potential mechanism underlying ID.

In 2016, Dr. Michele H. Jacob (right) at Tufts University developed a mouse model of ID, where  $\beta$ -catenin was deleted from the forebrain of a mouse. In this model, they found that when  $\beta$ -catenin was removed ( $\beta$ -catenin knockout), mice had more difficulty learning spatial learning tasks (see **Figure 1**) compared to mice with normal levels of ( $\beta$ -catenin) (Wickham et al., 2019). This is an example of **construct validity**, where an animal model is validated against what is known about humans with similar treatments (i.e., gene mutation). **Our job is to find out why** the  $\beta$ -catenin knockout mouse had poorer learning.



**Figure 1**

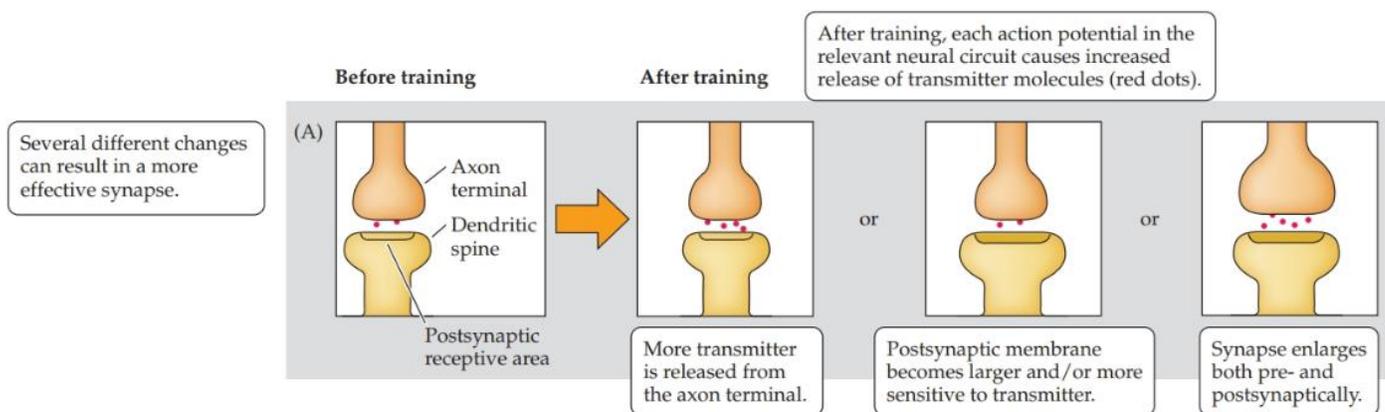
**Left:** Barnes Maze setup: a mouse is placed in the center of the maze (large circle) and their goal is to escape the maze. The maze exit is in one of the holes (small circles) that ring around the maze. Only one of these holes has an escape platform (red target circle), which can only be identified by crawling into it. The mouse can use spatial cues that are mounted outside of the maze (triangle, rectangle, star) to navigate the maze. The mouse eventually learns over multiple days or trials how to navigate the maze.

**Middle:** Data from the Barnes Maze comparing  $\beta$ -cat cKO mice to their wildtype littermates.  $\beta$ -cat cKO mice take much longer to learn how to escape from the maze compared to their wildtype littermates.

**Right:** Example trial on day 8 for a wildtype littermate (CTL) and a  $\beta$ -cat cKO mouse (cKO). Notice how many more stops the  $\beta$ -cat cKO takes before reaching the end marker.

## Identifying synapse strength by just looking at it

In class, we have learned that “neurons that fire together, wire together”, to mean that the more two neurons communicate with one other, the stronger the connection becomes (see **Figure 2**). Due to **long-term potentiation (LTP)**, the axon terminal and dendritic spine **increases** in shape and size. One consequence of this change in shape and size is that it is easier for the axon terminal to depolarize the dendritic spine and ultimately cause an action potential in the post-synaptic neuron. These kinds of synapses are **strong** synapses. This is the basis of learning, as learning new information will cause some synapses to strengthen, making it easier to “remember” (easier to depolarize dendritic spine, post-synaptic neuron action potential) the information stored at the synapse. This process serves as the way memories are formed. In contrast, strong synapses that are no longer needed will weaken via **long-term depression (LTD)**, which causes the axon terminal and dendritic spine to **decrease** in shape and size. This will make it harder for the pre-synaptic terminal to depolarize the dendritic spine and harder to cause an action potential in the post-synaptic neuron. These kinds of synapses are **weak** synapses. This process can be considered to be like “forgetting”.



**Figure 2**

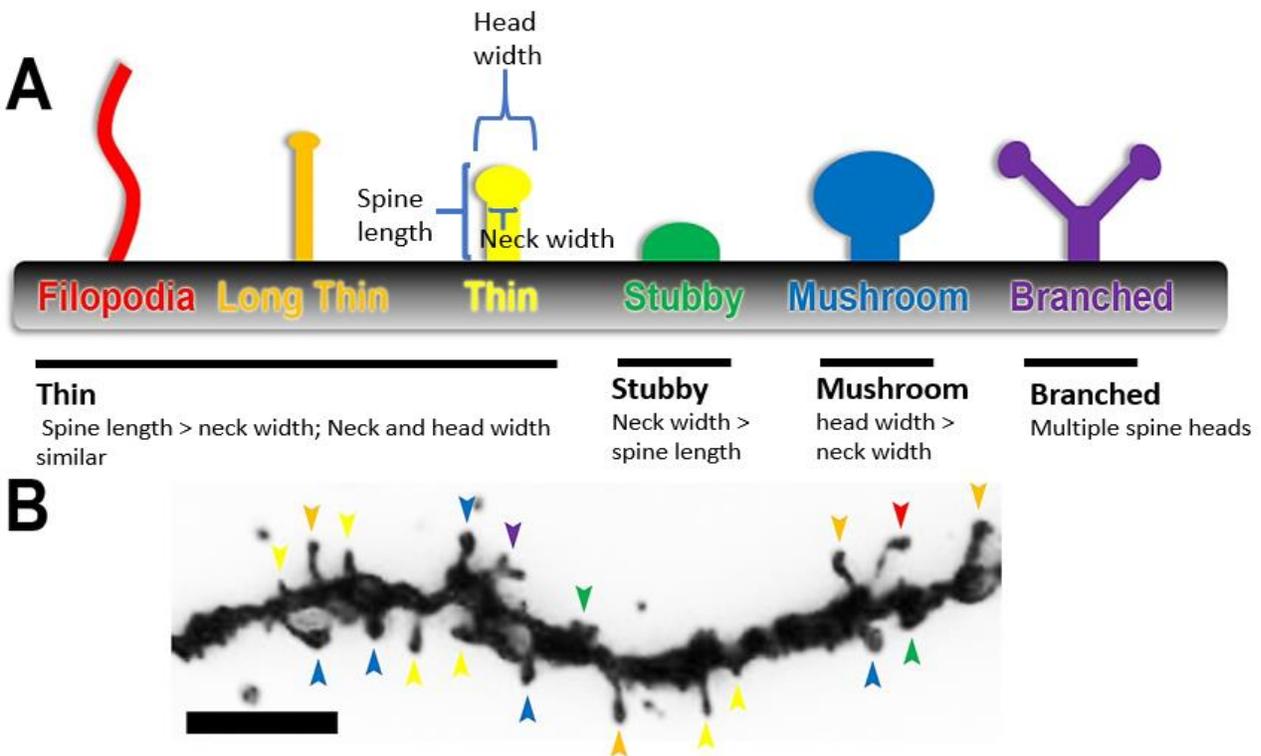
After training/learning, both the axon terminal and the dendritic spine grow in size.

What may surprise you is that we can typically tell if a synapse is strong or weak just by looking at the shape of the dendritic spine (**Figure 3**) (Risher et al., 2014). When a spine is born, it usually starts off very filopodial like with a very long neck and narrow head (called **thins**). After LTP, the spine **matures**, the neck retracts completely, and the head becomes bigger (called **stubbies**). After further LTP the spines neck extends from the dendrite and the head becomes even bigger (called **mushrooms**). Finally, the spine head becomes so big it needs to split, to make new connections (**branched**). This is the journey a spine takes from its weakest to strongest synapse forms. LTD can cause a transformation in the opposite direction, ultimately producing weaker synapses, sometimes eliminating the synapse completely.

Having lots of mature spines is not all that is cracked up to be, however. Imagine if most of your spines are quite mature. There is only so much room on a dendrite to make new spines and once a spine has reached peak maturity, it may be difficult to strengthen it more. It is not clear what will happen if a mutation or a drug causes synaptic spines to be very mature (and therefore, very strong). It may be that learning will be more difficult since there is no way to increase the strength of a synapse. It is maxed out—cannot get stronger. Thus, we have to be a little careful about equating having more synapses and stronger synapses as necessarily better for learning. On the flipside, having too few synapses or not being able to mature them, surely will cause problems for learning. If you cannot make synapses or make them more mature, learning cannot occur.

As an analogy, think about eating your favorite food when hungry versus full. When you are very full, eating something tasty will not really feel as good as when you eat that same thing when hungry. In a sense, when synapses are immature, they are “hungry” to grow and strengthen, as long as the conditions are right (wire, fire together). When synapses are mature, they are all full, and do not really want to eat (learn) anymore.

That being said, you can make the case that having too many immature synapses and too many mature synapses could lead to the same outcome—inability to learn.



**Figure 3 (Adapted from Risher et. al.,)**

A: Cartoon of spine maturation from weakest (left) to strongest (right)

- **Spine length:** how far the spine (neck and head combined) protrudes from the dendrite.
- **Head width:** the width of the spine head. Always as wide or wider than the neck.
- **Neck width:** the width of the spine that is not the head. Always less wide or as wide

B: Representative section of dendrite labeled as either Thin (red, orange, and yellow), Stubby (Green), Mushroom (blue), and branched (purple)

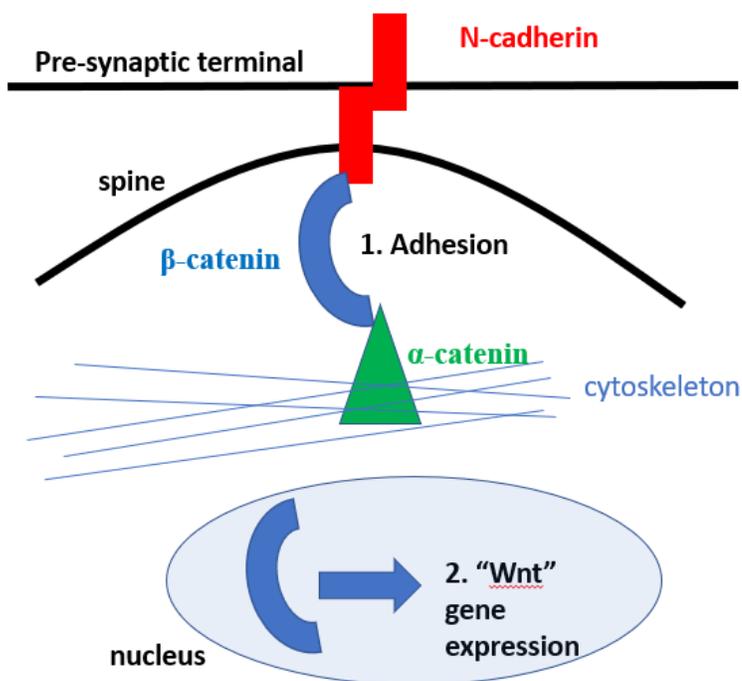
The Jacob Lab did a variation of Golgi staining called “lipophilic fluorescent dye DiI” which allows for fine resolution of synaptic spines. Due to the fine resolution, it is easy to both count the number of spines on a dendrite branch but also to classify each spine as above.

## Role of $\beta$ -catenin in the number of spines and the morphology of spines?

Synapses need **adhesion** proteins for them to form, maintain their stability, and ultimately for them to change morphology (i.e., thin  $\rightarrow$  stubby, etc). Adhesion molecules are proteins that keep the pre-synaptic terminal and post-synaptic spine physically linked to one another. One (*but not the only*) very important adhesion molecule is called **N-cadherin**. This protein is embedded in the membrane of both the pre-synaptic terminal and post-synaptic spine, and they “connect” in the synapse itself (see **Figure 4**). However, adhesion proteins need some kind of anchor to keep these connections stable. The anchor in neurons is the  $\alpha$ -catenin/cytoskeleton complex. Somehow, N-cadherin proteins must link to this anchor. This is where  $\beta$ -catenin comes into play. It binds both to N-cadherin and to the  $\alpha$ -catenin protein in the  $\alpha$ -catenin/cytoskeleton complex. Thus  $\beta$ -catenin serves as the stabilizing link between the adhesion protein (N-cadherin) and the anchor (the  $\alpha$ -catenin/cytoskeleton complex).

As an analogy, imagine your hands clasping each other, with one hand being the pre-synaptic terminal and the other being the spine. The N-cadherin would be your fingers interlocking.  $\beta$ -catenin are your hands and arms, and the  $\alpha$ -catenin/cytoskeleton complex is your body. Another analogy might be that the  $\alpha$ -catenin/cytoskeleton complex is the physical anchor that sets onto the bottom of the sea,  $\beta$ -catenin is the chain, and N-cadherin is the ship. In both analogies, without the anchor ( $\alpha$ -catenin/cytoskeleton) and linker ( $\beta$ -catenin), your hands or the

$\beta$ -catenin is called a “moonlighting” protein, meaning it often does multiple things at once. In addition to serving as a linking protein for N-cadherin and the  $\alpha$ -catenin/cytoskeleton complex, it can also enter into the nucleus and interact with the set of transcription factors called TCF and LEF. When  $\beta$ -catenin binds to these proteins, they increase the expression of a variety (~50 kinds) of Wnt genes (i.e., make more of the proteins that they encode) during learning. “Wnt” (pronounced “wint”) is a combination of the names of signaling molecules Wingless and Int-1, which are similar proteins found in flies where the Wnt pathway was first discovered. It is known that these genes play a role in learning (Maguschak & Ressler, 2011) as well as brain development (Mulligan & Cheyette, 2012).



**Figure 4**

$\beta$ -catenin has two major roles.

First, it helps link N-cadherin to the  $\alpha$ -catenin/cytoskeleton complex. This helps anchor the N-cadherin so that the synapse can stay stable, and potentially even grow.

Second,  $\beta$ -catenin can also enter the nucleus where it can cause increased expression of a category of genes called “Wnt”, which are typically important for learning and memory. In fact, learning something new can cause increases in gene expression of Wnt genes. How all of these genes spine density and morphology as well as learning and memory is still unclear.

The  $\beta$ -catenin cKO mouse lacks  $\beta$ -catenin, thus will not be able to bridge N-cadherin to the  $\alpha$ -catenin/cytoskeleton complex nor control Wnt gene expression.

## Project Overview: In class 11/5

You and a partner will team up to explore how loss of  $\beta$ -catenin will influence synaptic **spine morphology** and **spine density**, or the total number of spines on a dendrite per unit area. Spine density is a proxy for total number of spines, and spine morphology is a proxy for how strong each synapse is. Each of you will be given a pair of identical images. One image will be of a cortical dendrite from  $\beta$ -catenin cKO mouse, and the other one will be of a cortical dendrite from a control mouse that has normal amounts of  $\beta$ -catenin. You will count and classify the spines on each of these images. Thus, you will have spine density and morphology data from a single  $\beta$ -catenin cKO mouse dendrite and a control dendrite from the **prefrontal cortex**. The prefrontal cortex is a critical brain structure involved in learning and memory, and due to its complexity, is an area of the brain most likely to be perturbed by genetic mutations that influence neurodevelopment such as mutations in *CTNNB1*.

You will first individually analyze these data by yourself. Then, you will work with your partner to compare your data and make adjustments as needed. This is a very common practice in science. Double checking with a peer helps improve the accuracy of the data.

These data will be pooled together across the class (for a total of 20 cKO dendrites and 20 control dendrites). We will examine these data in a discussion in class and determine whether or not losing  $\beta$ -catenin influences spine morphology or spine density.

### A. Important Dates and Due Dates

**November 5<sup>th</sup>**: Overview and finding your data

**November 10<sup>th</sup>**: In class time for analysis

**November 11<sup>th</sup>**: Individual analysis due (3% of your grade)

**November 12<sup>th</sup>**: In class time for analysis with your group

**November 15<sup>th</sup>**: Group analysis due (3%)

**November 17<sup>th</sup>**: In-class discussion of data/Poster Presentation overview

**November 30<sup>th</sup>**: Upload video presentation to Canvas (12%)

**December 1<sup>st</sup>**: in-class time to watch presentations (2%)

### B. Hypothesis

- First, form a hypothesis about what you think might happen. Do you anticipate, based on your knowledge of  $\beta$ -catenin's functions, how the experiment will turn out? You must fill this information out and place this on your data analysis submission file (to be explained later)
- Hypothesis A**: The  $\beta$ -catenin cKO mouse will have (select one: higher, lower, the same) spine density than the control
- Hypothesis B**: The  $\beta$ -catenin cKO mouse will have spines that are (select one: more, less, just as) mature than the control
- For each hypothesis, you will want you justify *why* you think the results will turn out the way you predicted.

### C. Tips before you begin:

- The data analysis for these experiments can take a long time. I strongly encourage you to block out at least 1-2 hours per image. It may take longer, depending on how comfortable you are with PowerPoint or the analysis.
- As you go through and classify each spine, it will be important to save often
- If you have questions about what a spine should be classified as, it is OK to leave a "?" near it for later. You can confer with your partner on this or ask me in class.

- d. It is important to not rush through this—this is a real, live experiment. I have no idea what the outcome of the experiment will be. How careful you are will determine whether we can potentially find a remedy for this syndrome
- e. Do not procrastinate—as mentioned before, this can take time. It is worth your time. You are contributing to the betterment of people’s lives.
- f. Classification can be tough, but it always helpful to use the example image (**Figure 3**) as a guide to help keep you on track. Rather than using just the written definitions (see **section G** below), you may find it easier to just “visually match” what is shown on the example image.

#### D. Finding your data

- a. Go to canvas and identify the set of images (you will have *two* to work with) with you and your partner’s last name. It will be in the format of #\_LASTNAME\_LASTNAME\_A\_LENGTH or #\_LASTNAME\_LASTNAME\_B\_LENGTH.
- b. The # indicates the unique number identifying your image as unique from other groups.
- c. The letter (A or B) is a code for whether the image comes from a control or knockout animal. You will collect this data blindly. Blind data collection avoids any potential biases you may have.
- d. The LENGTH will indicate how long your dendritic segment is. This will be helpful in calculating spine density.
- e. For example, 52\_WICKHAM\_HAY\_A\_200 would indicate that it is Image 52, the group members are Wickham and Hay, the data comes from mouse A (could be a control or knockout), and the dendritic length is 200 micrometers.

**E. Viewing your image:** There are many types of software that you can use that probably are free or already on your computer. I **recommend** using Microsoft Powerpoint to view your image, just to keep everything consistent, and will make data sharing easier.

- a. Open Powerpoint
- b. Select “Insert”
- c. Select “Picture”
- d. Select one of your images
- e. You may wish to expand your image in Powerpoint, but be careful not to stretch it out so that it becomes too blurry
- f. You will want to use a drawing tool within Powerpoint to **draw small, color coded, arrows** to indicate the class of spine on your image (see below)

#### F. Annotating your image

- a. Color coding: you will want to use the following color arrows (**see more in section G: Criterion for Classifying Synaptic Spines**). Note—you may just use lines rather than arrows if you prefer, although arrows look more professional.

**Red:** Thin (this includes filopodial, long thin, and thin)

**Green:** Stubby

**Blue:** Mushroom

**Purple:** Branched

- b. Once you begin drawing on your image, you will want to
  - i. Save frequently
  - ii. **DO NOT MOVE THE IMAGE ON THE SLIDE**, as this will cause all of the arrows to no longer align with your image
  - iii. It will be very helpful to use the “zoom” function (bottom right of your screen) to get a better view of your spines.
- c. You will want to have a single powerpoint file with four slides (template on canvas).
  - i. **Slide 1** should contain your annotation for your file that has an “A” at the end.
  - ii. **Slide 2** should contain your annotation for your file that has a “B” at the end.
  - iii. **Slide 3** should contain your analysis Tables (see below).
  - iv. **Slide 4** will have your initial hypothesis (see Part B).

## G. Criterion for Classifying Synaptic Spines

**Thin:** the length of the spine is greater than the neck width, and the head and neck width are similar. Filopodia, Long Thin, and Thin will all be part of this category, and there will be no need to differentiate these three. This should be annotated with a **red** arrow.

**Stubby:** the neck width is similar to the total length of the spine. This should be annotated with a **green** arrow

**Mushroom:** the head width is much greater than the neck width. This should be annotated with a **blue** arrow.

**Branched:** spines with multiple heads. This should be annotated with a **purple** arrow. These are rare.

See **Figure 3** for a visual example and additional clarification.

## H. Analyzing your annotation

- a. Once you have marked each spine as one of the four classifications, you will want to count up each of different kinds of spines and enter the total number of each into the Analysis Table. You will also want to enter in this table the total number of spines (this is the sum of all four classification of spines).
- b. Calculate the spine %, also called spine fraction, for each spine classification. You can do this by taking the number of spines for a given classification and divide this by the total number of spines. Then, take this number and multiple it by 100.
  - i. **Example:** You calculated there are 32 mushroom spines on Image A, and a total of 120 spines in your image.  $32/120 = 0.2667$ , and  $0.2667 * 100 = 26.67\%$ . You will want to round to the nearest hundredth (second number after the decimal)
- c. Calculate spine density: You can do this by taking the total number of spines you measured and divide this number by the length of your dendritic spine.
  - i. **Example:** You measured 120 total spines on your dendrite, and your dendrite is 200 micrometers long. Therefore, your spine density is  $120/200$  or 0.60 spines/micrometer.

- d. You may enter all of this data on slide 3. You must do these calculations for both Image A and Image B.
- e. Each student within your group must analyze their data by themselves and upload this to Canvas by **November 11<sup>th</sup>, 2020. We will spend time in class on November 5<sup>th</sup>, 2020 and November 10<sup>th</sup>, to give you a head start as well as answer any clarification questions.**
- f. The file name for your powerpoint file should be #\_LASTNAME, where the # is identical to the # on your images.

**Analysis Tables, to be included on Slide 3 of your powerpoint.**

<b>SPINE MORPHOLOGY</b>					
<b>Image A</b>	<b>Total</b>	<b>% total</b>	<b>Image B</b>	<b>Total</b>	<b>% total</b>
<b>Thin</b>					
<b>Stubby</b>					
<b>Mushroom</b>					
<b>Branched</b>					
<b>Total spines</b>					

<b>SPINE DENSITY</b>		
	<b>Image A</b>	<b>Image B</b>
<b>Dendrite Length</b>		
<b>Total spines</b>		
<b>Spine density (total spines / dendrite length)</b>		

**I. Data verification**

- a. Once you have analyzed your data by yourself, you must set up a time to meet with your partner outside of class to compare your data. In this meeting, you must answer the following questions
  - Were there any major differences in your analysis? If so, identify the cause
    - a. Could it be errors in data input?
    - b. Could it be errors in how spine % or spine density were calculated?
    - c. Could it be differences in how you classified each spine?
    - d. Could one group member classify something as a spine and the other group member did not?
    - e. Please include this information on **Slide 5** of your new submission

Your goal is to agree on a classification for each spine. You may do this by analyzing the data together, going spine by spine (the ones you disagree on) and debate the morphology on each spine.

- b. Re-annotate (**Slide 1 and 2**) and re-analyze (**Slide 3**) your images.
- c. **Slide 5** will have your discussion question responses. To save time, it may be best to change the annotations on one of your partner's slides, rather than start over from scratch.
- d. **Slide 4** will have your initial hypothesis (see Part B).
- e. Each student must upload this file to Canvas on **November 15<sup>th</sup>, 2020. Each student must upload their shared file (i.e., you will submit the same document as your partner).**
  - i. The file name should take on the format #\_Yourlastname\_partner's\_lastname\_COMBINED.

#### **J. Data Discussion: 11/17**

- a. During this class, we will explore the results from the class as a whole
- b. We will focus our attention to trends in spine density and morphology between the control and cKO images
- c. Questions to consider:
  - i. Are there any differences between these variables?
  - ii. If so, how might these differences explain the behavioral phenotype of the mouse (learning impairment).
  - iii. If not, what could be some alternative explanations for why this mouse has learning impairments?
  - iv. What additional experiments might you conduct on the mouse to either confirm our results or in the case our results were negative (i.e., no differences between control and cKO)? What other hypotheses could we test given what we know about  $\beta$ -catenin's other functions aside from linking N-cadherin to the  $\alpha$ -catenin/cytoskeleton?

#### **K. Poster Presentation Guidelines**

Now that we have collected, analyzed, and discussed our data as a class, it is time to put these results into a communicable format. Sometimes, scientific products take on the form of lab reports, research articles, or presentations, but a very important form is the poster presentation. Poster presentations typically occur at scientific conferences that allow scientists to exchange ideas and data while also potentially forming collaborations. Effective poster presenting is critical for demonstrating your knowledge and skill as a scientist and has the potential to improve your job prospect.

Your group will each present a single poster, created in powerpoint (template on canvas), recorded via zoom, and uploaded via vidgrid. See the instructions below for more information.

##### ***Sections of your poster***

**Title:** states the question/problem that you are addressing, sometimes in question form

- Use bold typeface.
- Capitalize all first letters in each word in the title. Do not use ALL CAPS.
- Title should be short, meaningful, and eye-catching (no longer than two lines).
- Author(s) should be listed right under the title (Font size: **60**).

Font size: **70 - 80** for the title

**Introduction:** introduces your topic and briefly explains why your research is significant

- Place your topic within context of published literature. You should find at least 1 or 2 empirical or review articles (i.e., not news articles) related to either

- Synapse morphology
- $\beta$ -catenin
- CTTNB1 syndrome
- Developmental disorders
- Include just what is highly relevant. Minimize background information and definitions.

Max Wordcount: **100** Font size: Headings: about **44**; Main text about **32**

### **Objectives/Hypothesis:**

- State the experimental questions
- State your hypotheses (the expected answer to the experimental questions)
- Justify *why* you had your original hypotheses.

Max Wordcount: **50** Font size: Headings: about **44**; Main text about **32**

**Materials/Methodology:** tells readers what your research strategy was and how you actually carried it out

- Briefly describe your research methods and any equipment or software you may have used.
- You may also add figures, tables, flow charts, photographs, or drawings that describe your design.
- Here will be a great place to explain how you classified your spines

Max Wordcount: **150** Font size: Headings about **44**; Main text about **32**

**Results:** Data visualization and statement of findings. You will want three items in this section. Each item should be labeled as “Figure X” or “Table X” (X= is a number, starting with 1).

- Include the spine morphology and spine density tables you submitted as part of your final analysis (the one you did in pairs). You will want to adjust the size of these tables to fit nicely on the poster. Include a brief description (1-2 sentences) under the table, indicating what your individual analysis found.
- Include your annotated control and cKO images, with a brief description indicating what different color arrows mean.
- A bar graph, pie chart, some kind of graph of our class data, with a brief description indicating what our major results were when we combined all of our data as a class.

Max Wordcount: **50** Font size: Headings **44**; Main text **32**;

**Conclusions:** This is where you summarize your hypothesis and results.

- Focus on the main takeaway points
- Was your hypothesis supported?
- Are there alternative explanations to the findings?
- What is the significance of your findings?
- Identify at least one future experiment that could be conducted to better understand the role of  $\beta$ -catenin in the development of ID.

Max Wordcount: **300** Font size: Headings: **44**; Main text **32**

### **Works Cited**

- Throughout the poster, in-text citations should follow APA formatting: click [here](#) for a guide
- To list the works cited, use APA format again, click [here](#) for a guide.

Font size: **24**

**L. Presentation Guidelines:** You will record a presentation of your poster via zoom. See instructions below:

### **Zoom (screen capture + webcam)**

Zoom is a web conferencing tool that you have for free through your Etown login. You can host a Zoom meeting to record your presentation.

Log into your Etown Zoom account at <https://etown.zoom.us/>

1. Click Host a Meeting and choose Video On.
2. Click the Record button.
3. Choose to save to your computer.
4. Do your presentation.
  - You can share your screen to load your visual aids into the presentation.
5. End the meeting.
6. Your recording will be saved to your computer as an MP4. This will take several minutes.
7. You should upload to VidGrid (the college's video server) here: <https://use.vg/21Rmi4>
8. Click the copy button to copy the video link
9. Post the link to the video in your post.

### **[View the Zoom Tutorials](#)**

**Length:** The length of your poster presentation should not exceed 10 minutes

#### **Tips:**

- Do not read off the poster itself. A way of thinking about a poster presentation is that it is a powerpoint presentation with all of the slides visible at once, allowing you to go from slide to slide easily. You can refer to some of the text on the poster but the speaking portion of the presentation should be conversational
- Have one of the presenters share their screen with the poster opened in powerpoint.
- The text should be large enough to read off the poster during presenter view. Thus, there should be very little text overall. Short bullet points might be a better way to have text on the poster, and they can serve as your “talking points”.
- Speaking time should be equally allocated among group members. It should be clear that both members contributed to the project.

### **M. Discussion Posting Guidelines**

In addition to presenting a poster, you will be required to watch 2 other presentations and make substantive comments on the presentation. For each presentation, you should comment on the following:

- a. Is the data that they collected in line with the group data? If not, why not? If so, how so?
- b. How is the data they collected in line with the data your group collected? Compare and contrast the differences.

- c. Comment on the future experiment that was designed in their conclusion section. How might you expand on their idea?

**N. Rubric for poster and poster presentation**

Criteria	Exceeding (45-50)	Mastering (40-45)	Emerging (35-40)	Lacking <35
<b>Presentation of Research</b>	<p>Prominently positions title/authors of paper thoroughly but concisely presents main points of introduction, hypotheses/ propositions, research methods, results, and conclusions in a well-organized manner</p> <p>Narration and/or answering of questions is engaging, thorough, and adds greatly to the presentation</p>	<p>Contains title/authors of paper adequately presents main points of introduction, hypotheses/ proposition, research methods, results, and conclusions in a fairly well-organized manner</p> <p>Narration and/or answering of questions is adequate and adds to the presentation</p>	<p>Contains title/authors of paper presents main points of introduction, hypotheses/ propositions, research methods, results, and conclusions but not as sufficiently and not as well-organized</p> <p>Narration and/or answering of questions is somewhat lacking</p>	<p>Title/authors absent</p> <p>Does not sufficiently present main points of introduction, hypotheses/ propositions, research methods, results, and conclusions and is not well-organized</p> <p>Narration and/or answering of questions is lacking</p>
<b>Visual Presentation</b>	<p>Overall visually appealing; not cluttered; colors and patterns enhance readability; Uses font sizes/ variations which facilitate the organization, presentation, and readability of the research</p> <p>Graphics (e.g., tables, figures,</p>	<p>Overall visually appealing; not cluttered; colors and patterns support readability</p> <p>Adequate use of font sizes/ variations to facilitate the organization, presentation, and readability of the research</p>	<p>Visual appeal is adequate; somewhat cluttered; colors and patterns detract from readability</p> <p>Use of font sizes/ variations to facilitate the organization, presentation, and readability of the research is somewhat inconsistent/ distractions</p>	<p>Not very visually appealing; cluttered; colors and patterns hinder readability</p> <p>Use of font sizes/ variations to facilitate the organization, presentation, and readability of the research is inconsistent/ distracting</p>

	etc.) are engaging and enhance the text content is clearly arranged so that the viewer can understand order without narration	Graphics (e.g. tables, figures, etc.) enhance the text content is arranged so that the viewer can understand order without narration	Graphics (e.g., tables, figures, etc.) adequately enhance the text  Content arrangement is somewhat confusing and does not adequately assist the viewer in understanding order without narration	Graphics (e.g., tables, figures, etc.) do not enhance the text  Content arrangement is somewhat confusing and does not adequately assist the viewer in understanding order without narration
<b>Documentation of Sources, Quality of Sources</b>	Cites all data obtained from other sources. APA citation style is accurate	Cites most data obtained from other sources. APA citation style is accurate	Cites some data obtained from other sources.  Citation style is either inconsistent or incorrect.	Does not cite sources.
<b>Spelling &amp; Grammar</b>	No spelling & grammar mistakes	Minimal spelling & grammar mistakes	Noticeable spelling and grammar mistakes	Excessive spelling and/or grammar mistakes
<b>Comments:</b>				

## O. References

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