Daily Notes

August

August 24 (Lab Visit/Meeting)

Initial Meeting w/ Dr. Syed

- Discussed project ideas and we settled on Anesthesia in young children and it's effects on synapses formation and learning disabilities.
- Dr. Syed will set us up with Zainab Khan and Fahad Iqbal to work with us on this project
- Dr. Syed suggested reading a paper of his/other papers to gain background knowledge
- Gave us a brief tour of the lab
- Introduced us to Lily, a U of C student who is working on a similar project

August 30 (Class)

Intro to ASP class

- Spoke about her background as a researcher and scientist
- Spoke about mentor communication
- Discussed overall class expectations
- Mentioned future research proposal
- Told us about things to discuss with mentors

September

September 1 (Class)

Intro #2 to ASP class

- Showed us how to access some academic papers (NCBI, PubMed, etc.)
- Talked to us about citations
- Mentioned paperpile and informed us about set-up for the next class
- Discussed more class expectations and future assignments
- Talked to us more about meeting with mentors and overall timeline of the school year
- Set up Notion logbook and logged past days (Aug 24 and 30) as well as today

September 5 (Zoom Meeting)

Inquiry Meeting with Lily Koochak

Asked Lily questions about ASP and her experience with Dr. Syed

- Questions asked:
  - How does Dr. Syed's schedule work? Did he prefer you to come in during the ASP class or after school? (or does this depend on the students he has set us up with). Also, were you able to come in on weekends?
  - What can we do to really impress Dr. Syed?
  - What are his expectations from us?

    - Did he assign literature to read or did you find it on your own (also how did you learn more about the brain and your project)?
  - Answers:
    - He has no specific schedule, it's kind of random, come as needed.
    - Try to be prepared and be a curious student.
    - Listen to what he says to do and try to keep on top of your work.
    - He does not assign literature. Try to read up on things on your own, use google, watch videos, read papers and articles, read the AP Bio textbook. Don't be intimidated if you don't understand things right away.
  - Also gave us various resources to help us with our project (textbooks, and example of her poster, showed us her old logbook, etc.)

▼ ***September 6 (Class)***

Intro #3 to ASP Class

- Set up paperpile on computers and a mini tutorial of how to use it
- Dr. Garcia looks over logbooks to make sure they are ok
- Preparing for next classes meeting with Dr. Garcia
- Scheduled meeting with Zainab Khan (one of Dr. Syed's students) in the lab next week

▼ ***September 8 (Class + Check-in)***

ASP Work Class

- Check-in meeting with Dr. Garcia (all is going well)
  - Dr. Garcia said that we are on a good pace
  - Ask Zainab K. about Young persons in lab form and approval
  - Mentioned that Schedule B form can be completed at school and sent over to the lab (all other forms are handled between the lab and us/our parents)
  - Talked about the initial meeting with Dr. Syed and Lily Koochak
  - Showed Dr. Garcia the setup of our logbook
- Started reading and took notes on scientific paper "Review paper (detailed intro to the field - start here)"
  - This paper is by our mentor and one of the supervisors, a good intro to our project
  - Read and took notes on intro section (paperpile, background research section of notion)

▼ ***September 12 (Class)***

ASP Work Class

- Confirmed meeting with Zainab K. (Tosin missing French class, teacher approved)
- Continued reading and took notes on scientific paper "Review paper (detailed intro to the field - start here)"

▼ ***September 13 (Lab Visit/Meeting)***

2nd Meeting with Dr. Syed, Fahad, and Zainab K.

Asked Dr. Syed questions that we came prepared with

- Questions:
  - Ethics approval?
  - Training needed (Biohazard/Safety Course)?

    - Any forms needed or other approval (Schedule B Form)?
    - How often should we come?
    - Ask about research proposal (show rubric)
    - Young persons in Lab forms?
    - UCID?
    - What drug choice would be best?
- Answers:
    - No ethics approval needed for this project as the University has already approved it
    - No in-lab training needed, during experiments we will be always supervised and will be shown the right way to do things (However once the UCID is obtaned a Bioharard/Saftey course can be taken online)
    - No other forms from the lab needed (Schedule B is already doen by the school)
    - How many times we come into the lab is up to us and availability, howver he suggests that we should do things on weekdays rather than weekends. We will need at least one full day to design/perform the experiment (either miss a day of school or when we have a day off) and then all other times we will come in the afternoon for checking results
    - We went through research proposal togther (notes from that are shown below)
    - Young person in lab from not needed
    - Fahad will send us a link to help us get set up with a UCID
    - Sevoflurane is the most commonly used anesthetic among children and will be used fro our project

More notes from the meeting

- Dr. Syed wants us to read Nerea's review paper.
- Dr. Syed suggests that we should read review papers rather than study papers
- For the next meeting, we will have a timeline for the project made and our full schedule.
- For the next meeting, also have a short outline of our resreach proposal done
- When the rats have arrived, we will be contacted to begin the experiment
- They will be extracting the brain for us and then we can begin the project once the cell culture has been acquired.
- Dr. Syed introduced adding CBD oil (to test marijuana) as an aspect to the project.
- Dr. Syed introduced multiple exposures rather than just one because some kids get multiple surgeries and go under anesthesia a lot.
- Dr. Syed asked suggested maybe adding the neurochip to the project
- *We showed them our research proposal and we went through each aspect*
    - **Working Title:** "Comparative Analysis: The Effects of Anesthetics and Marijuana on the Brain"
    - **Abstract:** Research on humans is equivocal but on animals it i has very clearly shown that it has neurotoxicity, because of this we know that most surgeries require it so we need to find a safer way/ how it affects the brain so that we can put regulation in place and find certain methods/alternatives/ remedies or change the anesthetic drug so that it doesn't impact the brain in that way. We will also explore learning techniques to help these children. Most surgeries require anesthetics so we need to find a safer way. He also talked about how this affects kids that have multiple surgeries with anesthetics .
    - **Introduction:** Read more papers and then we will form this larger. Brain and synapse formation. The anesthetic can inhibit synapse formation. Especially in newborns or very young children who need. "cells

that fire together wire together". If they don't fire together they fire apart and cant connect. If they are loosing critical connections from birth, cannot develop those things later.

- **Objectives**: The first goal, how many cells die after exposure, we do this by adding a dye, we can physically see how many cells died. 2, see growth, measure diameter of cell at first and see how much it grew. 3, synapse connection, use screening to see how many connection were made. We will possibly expose the cells that survived a second time to see the effects on kids that have been impacted more than once. (We will clarify short term an long terms goals)
- **Variables:**
  - Independent variable: Sevoflurane
  - Dependent variable: Cell death, growth, and connections
  - Controlled variable: regular air
  - Confounding variable: Not really sure what this is, we may not have one (we will ask for clarification from mentor)
  - Independent variable (marijuana): CBD oil
  - Dependent variable (marijuana): Cell death, growth, and connections
  - Controlled variable (marijuana): methanol
  - Confounding variable (marijuana): Not really sure what this is, we may not have one (we will ask for clarification from mentor)
- **Questions or Hypothesis:** Are anesthesia and marijuana neurotoxic to human brains and how do they affect cognitive brain function?
- **Methodology:** (similar methodology to those in the scientific papers) Brain cultures exposed to variables, dead cells will be dyed and counted, growth of cells is measured by getting diameter before and after, synapses/connections between remaining neurons will be counted, second round of exposure and recount dead cells?
- **Significance:** This can help bridge the gap between children/people affected by the neurotoxicity of anesthetics or even prevent the negative effects of anesthetics on the brain
- **References:** (just our references)

▼ ***September 14 (Class)***

Dr. Garcia discussed the Research Proposal/ASP Work Class

Outline (example):

- Background Research:
  - Anesthetics/cognitive learning/synapse
  - Stats NA
  - Anesthetics - role in _____
  - Anesthetics interact with _____
- Research Question
- Goals
- Methods (variables)
- Significance (very important)

Tentative due dates:

- October 6th: (written) - have paper done at least a week before for professor and Dr. Garcia feedback
- October 11, 13, 17 (oral)

Class:

- Filled in meeting details from yesterday in the logbook
- Continued reading “Anesthetics: from modes of action to unconsciousness and neurotoxicity”

▼ ***September 18 (Class)***

Dr. Garcia discussed the Research Proposal/ASP Work Class

- Reviewed sevoflurane, how anesthetics work
- Find an answer: Why are we studying what we are studying?
- Continued reading “Anesthetics: from modes of action to unconsciousness and neurotoxicity”
- Dr Garcia spoke about the Research Proposal:

  Format: 12pt, double spaced, if using figures (they must have a legend)

  - Title: can be changed later and after more research
  - Intro: Outline, broader aspect and then narrow down to the research question.
  - Add to the field.
  - Must describe the need for our study, what was already studied?
  - Put the question after the intro
  - The goals are related to the research question (short/long term) “the goal of the study is to answer the following question _____”
  - Intro → research Q → Objectives (make the order make sense) must flow for our own project.
  - For our project what do we want to accomplish short/long term
  - Write variables with methodology or right before it
  - Confounding Variable: things you cannot control, diversity, there are variabilities within an individual you can’t control. Ex. rats being smaller than others. Thinks beyond our control yet they might influence our results. Ex. what a human did the night before the experiment.
  - Methodology is not marked highly because we are not currently fully knowledgeable yet, we must have a general idea, we must identify why we are using the methods we are using to answer the question.

▼ ***September 20 (Class + Check-in)***

Check-in with Dr. Garcia/ASP Work Class

▼ Updated Dr. Garcia about the project

- We are still reading and taking notes for background research and plan to start writing this weekend

▼ What we told Dr. Garcia

- CBD Oil has been added to the project as an element to be tested
- Told her about how we went through the research paper with Dr. Syed

▼ Dr. Garcia went through the year-long schedule with us

- This included when things were due/major deadlines/experimental setup etc.
- Year-Long Rough Timeline:
  - There will be writing assignments assigned throughout the year that will contribute to final paper
  - September/October
    - The majority of literature review/training.
    - Finalization of the oral and written Research Proposal (beginning/mid October)

        - Experimental Set-up (end of October)
    - Winter Break: December 21th - January 7th
    - November/December/January
        - Midterm Exams (Early January)
        - Experimental Work (End of October - November)
        - Experimental follow-up (November)
        - Finishing up Experiment (December/January)
        - Start data analysis
        - Write up of the Literature Review (Intro for the final paper)
    - February
        - Data Analysis
        - Poster Design/Oral Presentation
        - More literature review
    - March:
        - School Science Fair
        - Calgary Science Fair
    - Spring Break: March 23th - April 7th
    - April/May
        - Final papers finished and submitted
        - Final school presentations
- Emailed Fahad about literature for CBD oil since we cannot find much about it
- Made a plan for the Research proposal
    - Zz writes research proposal on anesthesia experiment (part a) and Tosin writes research proposal on CBD Oil experiment (part b)
- Searched for CBD oil papers

▼ ***September 22 (Class)***

ASP Work Class

- Read up on Synaptogenesis in the CNS: an odyssey from wiring together to firing together (Zainab)
- Found CBD Oil documents (Tosin)
- Set due dates for Research Proposal (Have rough draft done for both of them by Friday, Sept 29)
- Dr. Garcia talked about Logbook
    - Put September calendar and October calendar, very organized, classes must be shown on the logbook
    - Put tasks for each class/exactly what we did
    - Forward plan for October
    - Put all Check-ins and mentor meetings
    - Give Dr. Garcia permission to Notion
    - Put what we wanted to discuss in meeting on the logbook
    - Summary of Check-ins

- Email mentor, read literature etc.
- What we will do for each class of next month
- Put all emails in the logbook
- Strive for weekly meetings with mentor, meet or email.
- Logbook: organization, communication, schedule

▼ ***September 23 (Weekend Work)***

ASP Work Day

- Edited logbook to how Dr. Garcia described in class (add goals to schedule, update task)
- Zz started working on a draft of the intro of part a (sent this to mentor(s) for feedback)
  - Broke down the intro into categories for each paragraph
    - Exploring the Title's Significance and the Underlying Issue
    - The Historical Evolution of Anesthesia
    - An Overview of Anesthesia: Types and Mechanisms
    - Examining Sevoflurane: Advantages and Limitations as the Experimental Drug of Choice
    - Prior Research in this Field: The Importance of Our Study
  - Asked Dr. Syed regarding the methodology
  - Asked Fahad and Dr. Syed for feedback regarding the intro outline.
  - Link: https://docs.google.com/document/d/1e6AhgjGFKE_tlrxZoAzb41MKyb9Tckg4pUPQ0HKRets/edit?usp=sharing

▼ ***September 26 (Class)***

ASP Work Class

- Watched videos on “Daily Notes” section for background information
- Tosin continued reading papers on CBD Oil
  - Started to write outline of introduction on Part B of research proposal
- Emailed mentor for help with methodology section of RP, asked when we can meet next week to go over RP
- Dr. Syed gave us feedback on start to intro section of part B (refer to email)
  - Make more relatable to Highschool audience
  - Mention lack of research in the field, etc.
- Zz finished paragraph 2 for section A of the reserach paper (Different types of anesthetics and how each type works)
  - Began paragraph 3 of the introduction (Examining Sevoflurane: Advantages and Limitations as the Experimental Drug of Choice)

▼ ***September 28 (Class)***

ASP Work Class

- Tosin finished paragraph 1 of intro section part B (Introduction to Cannabis Legalization in Canada/The World)
  - Working on paragraph 2 of intro section part B (Background on CBD and Marijuana Compounds)
- Zz skimmed through a few articles and read majority of “Sevoflurane Exposure in Neonates Perturbs the Expression Patterns of Specific Genes That May Underly the Observed Learning and Memory Deficits”

- Zz worked on paragraph three of the introduction of the research paper.
- Sent the UC ID and Young Persons in Labs Forms to Dr. Syed and Fahad
- Updated Logbook with notes on Background Research
- Zz emailed (follow up) Dr. Syed regarding the methodology of the project.
- Zz asked Dr. Garcia about the parking permits at UofC
    - This will be too expensive rather, save the parking receipts and bring them in monthly.

▼ October

▼ *October 2 (Class + Check-in)*

ASP Work Class

- Dr Garcia Meeting:
    - New due date for written proposal is the 13th or 16th
    - Recommended that we should email mentors for meeting (sound urgent) w/ draft, tell them due date, can offer google meet if it is better
    - Send to dr garcia this Friday for paper feedback
    - Asked us how much we have done (just significance (Tosin), hypothesis, and methodology left)
- Broadly edited and proofread current draft of Research Proposal
    - Edited for grammar, phrasing, contractions, and added in sources
    - RP Drafting Document: https://docs.google.com/document/d/1e6AhgjGFKE_tlrxZoAzb41MKyb9Tckg4pUPQ0HKRets/edit?usp=sharing

▼ *October 4 (Class)*

ASP Work Class

- The City Science Fair has been pushed forward
    - Deadline for online portal: March 15th
    - Webber Academy Science Fair will be in early March
    - **City Science Fair:** April 18th-20th (Poster should be done)
        - If selected (12 projects), there is the CWSF in May
- Went over logbook comments that Dr. Garcia left and implemented changes
- Updated schedule to include meeting talking points for next meeting
- Decided that we will finish draft of research proposal (without methodology due to mentors) to send to Dr. Garcia by Friday for review
- Sent an email to mentors for urgent meeting for research proposal next week
    - Reminded him about how long it was since last meeting
    - We need to discuss research proposal and project progress
    - Scheldule meeting for next week (Monday-Wednesday for in-person)
    - Our due date for draft is middle of next week
    - Offered Zoom or Google Meet any day of the week for meeting
    - Attached research proposal draft for early viewing
- New tentative RP due dates:

    - Written: Oct 17th-19th
    - In-Class Oral Presentation: Oct 19th-Oct 31st

▼ ***October 6 (Class)***

ASP Work Class

- Added Dr. Garcia's suggestions to the logbook
    - Made entries more detailed according to her comments
- On October 11th Dr. Garcia will look at our logbook to ensure it is correctly updated
    - Show tasks and due dates in the calendar.
    - Show improvements
- Dr. Garcia spoke about logbook organization and basic tasks we should be doing in every ASP class
    - Update logbook (meeting notes, what you did during the class time)
    - Specific tasks on the major assignment that we have at the time
    - Communicate with mentors
- September logbook has been excused, next one is October w/ November tasks planned
- Advice from Dr. Garcia: Frequently ask Dr. Garcia if the logbook is up to her standards
- Drafted a follow-up email to Dr. Syed, will be sent Tuesday morning regarding a time to meet if he does not respond by the end of the weekend
    - Urged for a meeting as he has not responded to my emails this past week.
- Zz finished writing the short-term objectives for the objectives (part a) section of the RP
- Tosin finished writing the hypothesis (part b) section of the RP
- Only significance (for Tosin) and methodology is remaining to write
- RP Drafting document: https://docs.google.com/document/d/1e6AhgjGFKE_tlrxZoAzb41MKyb9Tckg4pUPQ0HKRets/edit?usp=sharing

▼ ***October 11 (Class + Zoom Meeting)***

ASP Work Class + Meeting w/ Dr. Syed

- Meeting with Dr. Syed re-scheduled as notice was not long enough.
    - Dr. Syed emailed us yesterday and said to pick a date + time and he will work around it
    - We replied with today's date and a meeting during class time, however he did not reply
    - We waited for 20 minutes in case he would join the meeting, but he did not
    - Scheduled and email Dr. Syed to try to find another meeting time (going out tomorrow morning)
        - Informed him that we were in the meeting and he was not there
        - Offered to meet again on friday, asked if this works for him
- Dr. Garcia gave us feedback on part of the RP that she has reviewed
    - Anesthesia section:
        - The history section needs to be shorted
        - The tone of the writing needs to be more formal rather than an "English class" tone
        - Terminology brought up in the hypothesis needs to be explained and explored in the introduction
        - Overall paper (mostly introduction) needs more depth

        - Is there a connecting factor between the two parts of the paper? If not, papers should be submitted separately
        - Part B is not yet reviewed
- BioRender: website for biological diagrams.
- Dr Garcia tip: make presentation simple and prioritize graphics over text
- Zz read pages 1-3 of the Brain Facts Textbook.
    - Annotations in “Background Research” section of Logbook
- Dr. Garcia’s Suggestions/Edits:








▼ ***October 13 (Class + Zoom Meeting + Check-in)***

ASP Work Class + Dr. Syed Meeting + Dr. Garcia Check-in

- March 4th is WASF
- Done project by mid-februry
- Only submit 2 things
- Dr. Syed Meeting (Very) Rough Notes:
    - Testing rats that are neonates p-0 (just born)
    - Checking after day 3, 4, 20 (how does it convert to human ages)
    - Keep between 10-15 pages
    - Sev is the most commonly used anesthetic bc of it quick induction and fast recovery, safer because it doesn’t irritate the throat (do not need to go in too much depth)
    - Marijuana as a recreational drug (describe what the research suggests, equivocal, so we need to further research this)
        - Use in mothers, young adults, contrast
    - Methodology:
        - neurons will be kept in an airtight chamber will be exposed to sevoflourane (equivalent to hoew it is in children) neurons will be put black on a incubator
        - use nerea or ryden’s paper (Dr. Syed’d students who have written papers that are similar to our topic, their papers are in the BG Research section)
        - cbd oil is not water soluble (usually kept in methanol)
        - andrew’s thesis (reword and rephrase it)

        - Include cell cultures, how to dissect and dissociate brain cells (how the culture dishes were prepared, kept in the incubator at 37 degrees)
        - Methanol (cells that are not exposed)
        - No notable confounding variable?
    - Significance: will provide insights in to the neurotixc affects of the agents on the brain and shed light on whether they affect it (future direction?) relate it back to the problem statement it would provide direct evidence to see if it would exert effects that can be
    - CBD Oil
        - The major component, talk about where it comes from.
        - Methodoly from Andrew, thesis form him fahad will send him a text
    - Cultures will be exposed for sevoflurane for 30 mins, cbd oil will be in culture dish for a day or 2 (its not known), cbd will be there and we can wash it to replace the media to wash it off but NEVER be able to fully wash it off
    - Short term objectives: look at cell death, does it really kill brain cells, does it affect their growth, does it affect connectivity
    - Controlled variables, all in the papers
    - We will test diff concentrations of sevoflurane?
    - Will be getting pups next week? Better methodology, preliminary data
    - For presentation: make a chart? add some pictures
    - Do many replicates of the experiment to have conclusive evidence ?
    - Sounds like we don't know the outcome of the experiment, no simple model that the effects have been tested.
    - mention using another agent, result in expanding data.
    - For presentation:
        - Tone it down to the level of grade, oversmart can backfire
        - You should be thinking about how CBD/sev causes cell death, how might that have happened (what are the gene and mechanisms involved? How do you know that this was not natural cell death?)
        - How did sevoflurane retard growth, mitochondira affects?
        - Learn about how cells die, grow, connectivity
    - Can lead to a bunch of neuro disorders (presecie connectivity patterns are important for the normal functions of the brain, it cannit be really tested in humans, it has been demonstrated that animals were hyperactive, had no learning or memeroy, using a novel approach to something that is not conclusively demonstrated, if a mother is smiking marijuana or taking drugs, it affects the babies for sure, rat model can show us what we cannot test in humans
    - Animals coming next week, we can come in after school for a late evening,
    - Send a reminder to Fahad to get his camera, some figures and drawings
    - Sure dr syed is in the lab, coordinate with fahad and make d, quick trip after school for pictures and methodology will be done
    - when schedules to do animals were do it all in the same day b
    - zainab k. coordinate with her and get started, writing will change, fahad will forward andrews thesis, lots of background Andrew.
    - send all the documents at once and @ karen

- Dr Garcia meeting:
  - discussed efficient methods to communicate with mentor rather than waiting days for a response.
  - See all notes above

▼ ***October 17 (Lab Visit/Missed Class)***

ASP Lab Visit

- Questions:
  - ▼ Q: MAC
    - ▼ Answer: Measures the potency of an anesthetic in your blood system.
  - ▼ Q: Confounding variable
    - ▼ Answer: Sex of rat, brain trauma.
  - ▼ Conversion of rat age to human age.
    - ▼ Answer: Calculations online
  - ▼ Q: Is just the anesthesia administered or what supplumental drugs are added. Propofol for pain.
    - ▼ Answer: In real life it's with a combo of drugs with the anesthetic. Judges will ask how its a real representation, we only test sevoflurane cause if we do a combo how will we know the root cause is if we test everything at once, sevoflurane is the most commonly used anesthetic. Sevoflurane has fast induction and quick recovery. Other drugs are added so that the patient is not in pain after, Sevoflurane makes the patient uncouncious. Supplemental drugs add to muscle relaxation.
- Dissected the rat pups (Sprague Dawley)
- They are P2 (2-days old)
- The study will utilize Sprague Dawley rat pups aged 2 days (P2), with 3 pups used and their hippocampus tissues cultured in 24 dishes, adhering to ethical animal care guidelines.
- The experiment involves brain dissection, including the preparation of digestion solution and cortical culture medium, as well as the dissection process with careful steps to preserve the brain.
- Cell dissociation includes the use of triturating pipettes, digestion solution, and incubation, followed by a series of washes and trituration to achieve a consistent culture medium.
- Plating cells into 2 mL dishes involves a careful process of adding the cell suspension to the plates, allowing an incubation period for cell adherence, and routine media changes to support cell growth and proliferation.

▼ ***October 19 (Class)***

ASP Work Class

- Sent out an email to Zainab to confirm the meeting for tomorrow:
  - Email: "Hi Zainab, I'd like to confirm that Tosin and I will be visiting the lab tomorrow after 11:45 AM to check on the progress of the brain cell cultures."
- Finalized the Research proposals (still missing methodology)
- Zz and Tosin read over each others research proposals and revisions have been made

▼ ***October 20 (Lab Visit)***

ASP Lab Visit

- We are no longer testing sevoflurane, we are now testing ketamine
  - This will change a lot of things in the research proposal
- Learned how to use the microscope:
  - Turn on the computer

    - Hold the shutter button on the left and while holding, turn on the red switch until you hear 2 beeps then release.
    - Once the computer is on, click “zeiss”
    - Open the application “Zeiss”
    - There will be two options choose “Zen Pro”
        - Image Processing: This is for already captured images when you want to analyze them.
        - **Zen Pro:** To take new images
    - Go back to the microscope:
    - Adjust the focus, (distance of object from specimen) this can be done on the device near the computer or on the actual microscope itself
    - Move the specimen on the application or using the joystick.
- We are using a Zeiss: Wide Fixed Microscope
    - Use this to record activity and look at cells
    - This microscope has an incubator set up
        - This works the same way as the main incubator (95% oxygen, 5% co2)
- When imaging, must be quick as the cells can’t be outside of their incubator for too long. (once in the ‘incubator of the microscope its okay)
- When changing the lens, be very careful because if you don’t move the stage, the rotation of the lens can crush the lens.
- Take images and save it to our file called (”Zainab & Tosin”)

▼ ***October 23 (Class)***

ASP Work Class

- Dr. Garcia spoke about the Oral Research Presentation
    - Punchline (1 minute sums up the goal of the experiment)
    - Start with your title, if it is complicated and hard to understand, break down the meaning of it
    - Couple of slides for background (2-3 slides)
    - Research question + aims/hypothesis?
    - Methods (flow diagram of methodology) (2-3 slides)
        - Include variables (1 slide)
    - Significance
        - from the beginning, must explain the significance and bring it back to the punchline
    - Ask Dr. Garcia: What order should we do the RP oral presentation?
    - Style of Presentation:
        - graphics/visuals instead of a lot of text
        - if we have text, must be large font but small amount of text
        - make sure to have engagement with the audience
        - loud voice, dont be bland, be engaged
        - Q-cards with just quick notes on what i will talk about (don't look at cards during presentation)
        - the flow is very important, follow a logical path

        - no need for a title slides for each topic
        - PRACTICE IS KEY
        - dont use a template, just a white background
    - Worked on methodology:
        - Tosin and Zz will have the same methodology up until the exposure of (ketamine, CBD Oil)
    - To condense all the info in ten minutes:
        - Get rid of in-depth details
        - Present the bare minimum to relay the basic info for the project.
        - Condense: quality>quantity
    - Research proposal due by October 31st
    - We will present on November 6th
    - For oral presentations that you are watching, you only need to be present for 1 day (3-4 presentations) and ask questions; the more presentations you attend, the better your presentation can be
    - Dr. Garcia says RP will be synthesized now instead of separate
    - Dr Garcia's advice for intro format: general stats → medical use → recreational use → mechanisms of action

▼ ***October 25 (Class)***

ASP Work Class

- Dr. Garcia spoke more about the RP Oral Presentation:
    - 10 minutes = 10-12 slides
    - Example
    - 1: title/punchline
    - 2: main idea
    - 3: main idea
    - Research Question (1/2 slides)
    - Methodology (flow diagram explaining the rationale for the experiment) answers: how is my methodology allowing me to answer my research question (1-4 slides)
    - Finish off with significance (bring up the significance RIGHT at the start, why are we doing this?)
- Logbook and written proposal due on October 31st
- Mentor Evaluation at end of October
- November 6: Oral Presentation
- Dr. Garcia spoke about LogBook:
    - Calendars for November
    - Task
    - Reflections for 90 minute ASP Classes
- Meeting with Dr. Garci:
    - Back to 1 proposal
    - switch to ketamine instead of sevoflurane
    - Oral presentation that is a bit longer, about 12 minutes
        - background on ketamine and CBD Oil

    - 💡 Methodology and significance combine for the OP
    - Combined punchline (clinic and recreation use and the potential for harm in both cases)
    - stats and mode of action
    - punchline: explains the title and is a hook (answers WHY question, why are we studying this so some significance)
    - next, background information so that people understand and have a general idea.
    - No later than this Friday, send Dr. Garcia the research proposal draft.
- Quick IDEA: for our experiments, we could compare cell cultures given with medical dosages vs theoretical recreational dosage to compare cytotoxic effects in a medical setting vs a recreational setting
- Quick IDEA: Compare ketamine and cbd oil, same functions and effects on humans, which one is better?

▼ ***October 27th (Class + Lab Visit After School)***

- Worked on synthesizing both research proposals (intro done in class, rest at home)
- General Plan:

Ketamine & CBD - HP Cultures Project.pdf

- **INTRO:**
    - The research proposal aims to investigate the effects of marijuana (using cannabidiol oil) and ketamine on neurons, particularly in individuals with developing brains, addressing a critical knowledge gap.
    - The brain's development in early life is crucial for cognitive growth, and the effects of anesthesia on neonates' neurological development have not been extensively studied.
    - Both ketamine and CBD oil interact with the brain's intricate system of receptors and neurotransmitters, impacting various neurological functions.
    - Ketamine is used as an anesthetic but also recreationally, with potential for short-term and long-term side effects, while CBD oil is being explored for its therapeutic potential, including pain relief and anxiety reduction.
    - Studies have shown that marijuana and CBD can have negative effects on memory, behavior, and neuronal function, particularly during critical stages of brain development, suggesting potential risks associated with their use.
- **Research Questions:**
    - How do ketamine and CBD oil exposure affect neuronal cell viability?
    - What effect does the exposure of ketamine and CBD oil have on neuronal growth?
    - How does the synaptic connectivity of neurons exposed to ketamine and CBD oil compare to those that are not exposed?
- **Hypothesis:**
    - Exposure to ketamine and CBD oil for 1 hour in rat brain cultures results in decreased cell viability, cell growth, and fewer synaptic connections, primarily due to ketamine's interference with these processes.
    - Previous research indicates that CBD can also negatively impact neurogenesis and synaptogenesis, contributing to declines in cell viability, cell growth, and synaptic connectivity in the brain cultures.
- **Objectives**:
    - Short-Term

        - Quantify how many cells survive and die after their exposure to ketamine and CBD oil
        - Measure how much ketamine and CBD oil can change neuronal cell growth
        - Assess how the creation of synaptic connections are affected after exposure to ketamine and CBD oil
    - Long-Term
        - Explore the effect of ketamine and marijuana on the brains of people who use it (cognitive function and brain health), especially youth and nursing mothers
        - Investigate whether there will be everlasting consequences on cell growth and synaptic connections in the hippocampus or other brain regions
        - Examine the mechanisms of action by which ketamine and marijuana affects the brain in order to advance other studies in this area
        - Explore alternatives to ketamine and marijuana in the medical field that may be less harmful to patients' brains
- **Methodology**: use procedures they sent us
    - The study involves the use of 2-day-old Sprague Dawley rat pups for experimentation, with a total of 3 pups and their hippocampus tissues cultured in 24 dishes, adhering to ethical guidelines for animal care.
    - The process includes brain dissection, cell dissociation, plating cells into 2 mL dishes, and administering CBD oil and ketamine into the cells, with careful calculations and steps to ensure the desired concentrations and uniform distribution of the substances for subsequent research or analysis.
    - Quantification is still missing, emailed Fahd about it
- **Variables:**
    - Independent:
        - Ketamine and CBD oil exposure; whether or not a brain cell culture will be exposed to the substances.
    - Dependent:
        - Cell viability, cell growth, and synaptic connection.
    - Controlled:
        - Rats used will all be from the same litter (same mother, same age)
          Conditions that brain cell cultures are kept in in the incubator will be consistent: same CO_2 levels, same O_2 levels, same temperature, and same humidity. Brain cell cultures will be exposed to either ketamine, CBD oil, or both, for the same duration of time
- **Significance:**
    - Studying the effects of ketamine and marijuana on the brain is crucial for medical and real-world applications.
    - This research has significance in neuroscience and pharmacology due to the increasing interest in their impact on brain function, especially on neurons.
    - Understanding the neurological effects of these substances can lead to improved medical treatments and harm-reduction strategies.
    - It is essential for public awareness and education about the potential consequences of using ketamine and marijuana.
    - The research aims to shed light on the mechanisms of action of these compounds, informing future medical and regulatory decisions regarding their use.

▼ Zz went to the lab, Tosin could not come

- Complete live/dead assay

- Imaged all 24 cell cultures before/after adding the dye
- Fixed the cells
- Dish number 3: Zainab Khan crushed cells
- Dish number 12: something went wrong (they did not clarify)
- 6.29 microliters CBD oil
- 10 microliters ketamine
- Some cultures has both
- Fixed cells using pdh (has to be handled by 18+, used under the fume hood)
- Pbs saline used

▼ ***October 31st (Class)***

ASP RP Presentations/Work Class

General Presentation Notes:

- Can have some designs
- Statistics (significance)
- Diagrams (introduce aspects of the title (why/how we are using, what we are doing with these))
- Why this study? Why ketamine, why CBD oil
- How, research questions
- Few short term, 1 long-term goal
- Introduce variables
- Drew a diagram on the whiteboard
- Methodology in a flow chart format
- Simple title that people can understand
- With the flow chart, if we have space we can add images to explain it better
- Explain what is in our brain
- explain a synapse, neuron
- Talk about medical/recreational use
- write about staining, immunohistochemistry
  - green and red, explain that
- fluorescent microscope
- talk about the mentor’s previous studies
- put captions under images (citations), lots of images
- every vocab/word we put we must know everything about.
- prepare for Q,
  - why rats?
  - why hippocampus?
    - previous studies have shown…
  - how does this reflect real life?
  - why are you putting the CBD and ketamine in the culture, why aren't you exposing a live rat?

    - the rate at which effects happen? (ask mentors)
    - how do synapses form in a cell culture if there is no consciousness?
    - why do u break up the synapses to make the culture?
    - How does immunohistochemistry work
    - how does this relate to IRL
  - Baby the presentation down and don't use big words (but still sound smart)
  - Don't put any results
  - Why this technique
  - Look at our presentation from the perspective of someone in our class.
- Cooper's Presentation:
  - Questions:
    - Why the cortex? Why are you taking full slices?
    - Why are you using brain "slices" rather than cultures?
  - Changes in complement protein deposition and its role in.
  - studying effect of concussions on the brain
  - Slide 1: intro - explaining the title/what a neuron is
  - Slide 2: intro - stats about concussions in kids, and what concussions to to meurons/the brain
  - Slide 3: intro - what is microglia? role in synaptic pruning
  - Slide 4: intro - what is the complement system?
  - Slide 5: intro - previous studies
  - Slide 6: research question and objectives
  - Slide 7: variables and hypothesis
  - Slide 8: methodology
  - Slide 9: methodology pt. 2
  - Slide 10: methodology pt. 3
  - Slide 11: significance
  - no further questions, very well-presented project, an interesting topic!
  - feedback for dr. g: speak slower, explain more
- Jessica's Presentation:
  - Targeting autism-risk genes through knockout of those genes
  - Slide 1: intro - statistics of autism
  - Slide 2: intro - crispr/cas9 and why it is being used
  - Slide 3: rationale/research question
  - Slide 4: objectives (short-term/long-term)
  - Slide 5: variables
  - Slide 6: methodology (overview and diagram)
  - Slide 7: methodology pt.2 (flowchart)
  - Slide 8: methodology (further details)

    - Slide 9: Significance
    - Slide 10: thanks!
    - no further questions, very well-presented project, and an interesting topic!
    - feedback from dr. g: more pictures?
- Discussed the RP with Dr. Garcia, general notes are that we need to condense things and reorder them to achieve better flow
- Discussed the methodology and the rest of RP after school, same general notes, overall good RP
    - RP's due date is Monday
    - The oral presentation was moved to Nov 8th if needed the 15th

▼ November

▼ ***November 2 (Class)***

ASP Work Class

- **Long term-Plans:**
    - **Literature Review:** Dec 15th
    - Early February: Experimental procedures or methods section.
        - Practice Oral Presentation
    - Early March: Ready for Science Fair
    - End of February/beginning of March: Results
    - April: Analysis (put the entire paper together)
    - May: Final Paper
        - What do we know from the literature to explain the results?
- Dr. Garcia talks about the literature review:
    - Once we are done research proposal and oral proposal we can work on the Literature review.
    - It is an introduction for the final paper
    - Contains all background research we have done for the research proposal
    - Must have depth and flow well
    - If you get no feedback on the intro of the original RP, you can copy and paste and submit it
    - Apply all edits from the original RP Intro into the literature review
- Final Research Paper due at the end of May (Heaviest weighted assignment)
    - Intro (Literature Review)
        - December 15th
    - Methods/Experimental Procedure
        - Early February
    - Results
        - End of February/Beginning of March
        - 2nd oral presentation (practice for SF)
    - Analysis
        - April

- Acknowledgements/References

- Class Work:
  - Continued editing Dr. Garcia's notes for the Research Proposal:
    - Edits: https://docs.google.com/document/d/1m460hllfUB-cG78YLclybIN6Wo-S386je7dHIS-OZCc/edit?usp=sharing

▼ ***November 6 (Class)***

ASP Work Class

- Revised Dr. Garcia's edits
  - Hypothesis revised (just one edit)
  - Made the small edits to the objectives, variables, significance section
  - Added the Quantification section from the methods that Fahad sent and overall methodology was edited

After School

- Re-formatted and condensed the intro of the research proposal
  - First paragraph kept the same.
  - Condensed and combined hippocampus info and neuron info.
  - One paragraph each for modes of action, rec/med use of CBD and Ketamine
  - One paragraph for effects on neonates
  - Final section is on previous research
- Read over the whole research proposal and made final revisions, submitted the assignment and GC and turnitin

▼ ***November 8 (Class)***

ASP Research Proposal Presentations (Mariska, Elliot, Owen, Natalie)

Participation:

- Cooper (each asked a question)
- Owen (each asked a question)
- Mariska (each asked a question)
- Natalie (Tosin asked a question, Zz was not here)
- Elliot (Tosin asked a question, Zz was not here)

General Notes about Presentations:

- Diagrams/visuals to help them understand is great
- Most people are one minute under the time limit
- All the processes know immunochemistry well and general idea of the formulas
- For us: why are we using Sprague Dawley rat?
  - how to apply this to humans
  - we are trying to understand the molecular mechanisms
  - at a cellular level, takes years of research

Presentations:

- Owen's Presentation:
  - Use of neuronal networks to identify heart diseases using ECG

- Slide 1: Electrocardiogram (diagram, what it is)
- Slide 2: Acute Coronary Syndrome (heart disease, abnormal blood flow to the heart)
  - explains it
- Slide 3: Arrhythmia (heart disease, irregular electrical signals)
  - explains different waves and what they mean
- Slide 4: Convolutional Neuronal Network (flow chart explaining it, detects patterns inside the ecg)
  - input
  - convolutional layer
  - pooling layer
  - fully connected layer
  - kernel
- Slide 5: Research Questions
- Slide 6: Methodology
- Slide 7: Significance
- Slide 8: Thanks
- **Questions:**
  - How will you make sure that this technology can be trusted on human patients? (how will you mitigate tech concerns?)
    - A doctor will use it to supplement them
  - How are these ECG readings kept controlled (where did you get it from, how will they take into account human differences?)
    - The differences aren't taken into account hearts b/c the heart works mostly the same for most people

- Mariska's Presentation:
  - Effect of consuming a lot of fat and sugar on muscle health
  - Slide 1: Diet and Obesity
    - causes hypertension, stroke, heart attack, diabeties, depression
  - Slide 2: Inflammatory System
    - cytokines, mcp-1, macrophages, cd68+
  - Slide 3: Previous Studies
  - Slide 4: Previous Studies Pt. 2
  - Slide 5: Research Questions
  - Slide 6: Variables
  - Slide 7: Methodology
  - Slide 8: Methodology Pt. 2
  - Slide 9: Significance
  - Slide 10: Thanks
  - Questions:

        - Why haven’t you explored other muscles, why do studies just explore vastus lateralis and soleus muscles (leg muscles)?
            - They are testing 2 different types of muscles in the body (from the legs) which are the two main types of muscles overall in the body
        - Why are previous studies conducted in male rats?
        - Why did the females have a higher intramuscular fat infiltration?
        - What happens to the muscles when they are infiltrated with fat? What happens to the body long-term?
        - Why are you choosing to use rats to test this? How will your findings relate to humans?
            - The muscles are similar to humans and respond in a similar way, previous studies have used this model.
- Natalie’s Presentation:
    - The effect of compression garments on heart rate variability in patients with POTS
    - Slide 1: What is POTS
        - Blood does not get pumped back up into body when patients are standing or sitting down
        - heavily affects women vs. men (91% vs. 9%)
    - Slide 2: Heart rate variability
        - amount of time taken between each heart beat
    - Slide 3: Compression garments
        - act like the autonomic nervous system
    - Slide 4: What we know/don't know
    - Slide 5: Objectives
        - using MiniP MATLAB program
    - Slide 6: Hypothesis
    - Slide 7: Variables
    - Slide 8: Study Flow
    - Slide 9: Methodology
        - data collection, put it through the program
    - Slide 10: Significance
    - Slide 11: Methodology
    - Questions:
        - How will you get human subjects? Will you factor in the fact that it affects women more?
            - Not really, because it affects women and men the same way
- Elliot’s Presentation:
    - Autism affect on brain? Did not understand the title and it was not explained
    - Slide 1: Background
    - Slide 2: Background Pt. 2
        - Gut-brain axis, 3 ways of communication
    - Slide 3: Background Pt. 3
        - Microglia, what is autism?

- Slide 4: Objectives
  - how c. innocuum strains on social behaviour and microglial/neuronal function
- Slide 5: Question/Hypothesis
  - Is c. innocuum a pathogenic bacteria that afftecs..., yes it will
- Slide 6: Variables
- Slide 7: Methodology
- Slide 8: Methodology Pt.2
- Slide 9: Methodology Pt. 3
- Slide 10: Significance
- Slide 11: Thanks
- Questions:
  - How do you know that sociability is actually linked to autism and not just personality?
    - They are using 10 different mice to try to mitigate this variability.

- Sent an email to the mentor:
  - Research Proposal successfully submitted
  - Project presentation to the class on November 15th
  - Request to discuss plans for the experimentation phase
  - Scheduling a meeting for coordination

▼ ***November 15 (Class + Presentation Day)***

ASP Research Proposal Presentations (Me and Zz, Brynn)

- Morning before class: Practiced presentation 2X (It was around 12 minutes long)
- Note from presentation: Make sure to deeply understand live dead assay and immunofluorescent staining/immunohistochemistry/immunocytochemistry
- **Our Presentation:**
  - Questions Asked:
    - Why test CBD vs Ketamine?
    - Immunofluorescent/histochemistry?
    - Which protein?
    - Is there a limit for CBD where it is good?
    - Clarify methodology
    - Etc.
  - We need a punchline (we had it)
  - Too many words/info
  - Speaking a lot slower
  - Try not to read from the slides, its better to cut things out and explain rather than reading all the info; this can be to your detriment in science fair (with more time we will know the project more)
- **Brynn's Presentation:**
  - Climate Change & Biodiversity
  - Decreased biodiversity, fewer microbes we are exposed to.

- increased co2 levels, increased photosynthesis
- drought
- Synthetic biology can be used to prevent climate change effects on plants
- Genetic diffs between an endangered and non-endangered pine species
- Methodology, maps
- Question: What does previous research say about the genetic differences?
  - There is not a lot of research into genetic differences but there are into phenotypical differences which is part of why this project is necessary

▼ ***November 16 (Lab Visit)***

ASP Lab Visit

- Followed pt. 1 of the immunocytochemistry procedure:

IMMUNOCYTOCHEMISTRY Nerea Jiménez Téllez (Syed's lab)

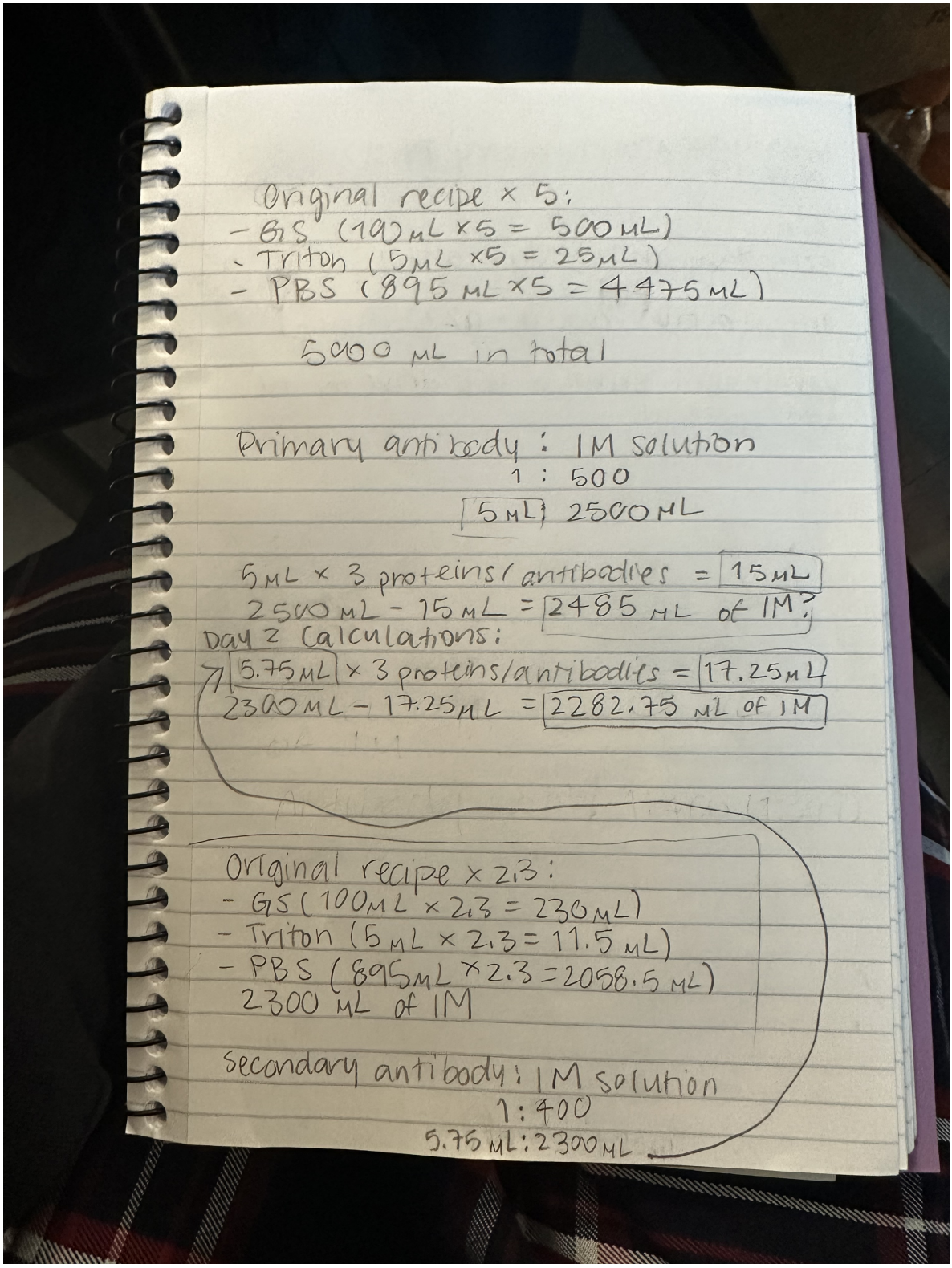
Immunostaining Protocol

1) Check MOWIOL, ask for more if needed
2) Take out all the media from the dish (Use pipette to remove all culture medium)
3) Wash 3x with PBS (take out full 1 mL, add 1mL) – 10 min wait times
4) Block 1 hour at RT with 0.1 % Triton X with 10 % goat serum (IM) – 100 µL per plate
In this step you can stop here if need for 1 hour
5) Remove all IM solution from the well
6) Prepare primary antibody solution 1:400 diluted in IM
7) Add 100 µL of primary antibody solution per dish
8) LEAVE OVERNIGHT AT 4 DEGREES

Solutions

	10 X PBS	**Incubation media for 1 mL**	
Na_2HPO	12.7 g	10% Goat serum	100 µL
NaH_2PO_4	2.65 g (2.28 g NaH_2PO4)	0.5% Triton x100	5 µL
NaCl	85 g	1 X PBS	895 µL
dH_2O	to 1 L, pH to 7.4 after dilution	[redacted]	

- Notes:


Original recipe x 5:
- GS (100 mL x 5 = 500 mL)
- Triton (5 mL x 5 = 25 mL)
- PBS (895 mL x 5 = 4475 mL)
5000 mL in total
Primary antibody: 1M solution
1 : 500
5 mL : 2500 mL
5 mL x 3 proteins/antibodies = 15 mL
2500 mL - 15 mL = 2485 mL of 1M
Day 2 Calculations:
5.75 mL x 3 proteins/antibodies = 17.25 mL
2300 mL - 17.25 mL = 2282.75 mL of 1M
Original recipe x 2.3:
- GS (100 mL x 2.3 = 230 mL)
- Triton (5 mL x 2.3 = 11.5 mL)
- PBS (895 mL x 2.3 = 2058.5 mL)
2300 mL of 1M
Secondary antibody: 1M solution
1:400
5.75 mL : 2300 mL

▼ ***November 17 (Class + Check-in + Lab Visit)***

ASP Research Proposal Presentation (Vincent)

- **Vincent's Presentation:**
  - Determining the effectiveness of viruses to see if they are able to kill bacteria (e-coli)
  - Multi-Drug Resistance
  - Bacteriophage
  - MDR bacterial infectious diseases are prevalent
  - Research questions
  - Objectives
  - Hypotheses
  - Variables
  - Methodology
  - Significance

ASP Check-In

Notes:

- We're not doing which substance yields better results anymore
- This is the end of the experiment
- Analyze and imaging left
- Study the technique
- Why do we use each animal proteins
- What does each protein show (what is teh cloour of teh flourencencce)
- How to use this experiments to answer our questions
- Secondary proteins should be the same animal or different?

ASP Work Class

- Filled out the CYSF Forms on the website
  - Basic Project Info
  - Ethics Due Care 2A
  - Hypothesis
  - Research
  - Variables
  - Declaration
  - Procedure will be filled out later because we may need to make edits
- Completed all Science Fair documents for the Google Classroom

ASP Lab Visit

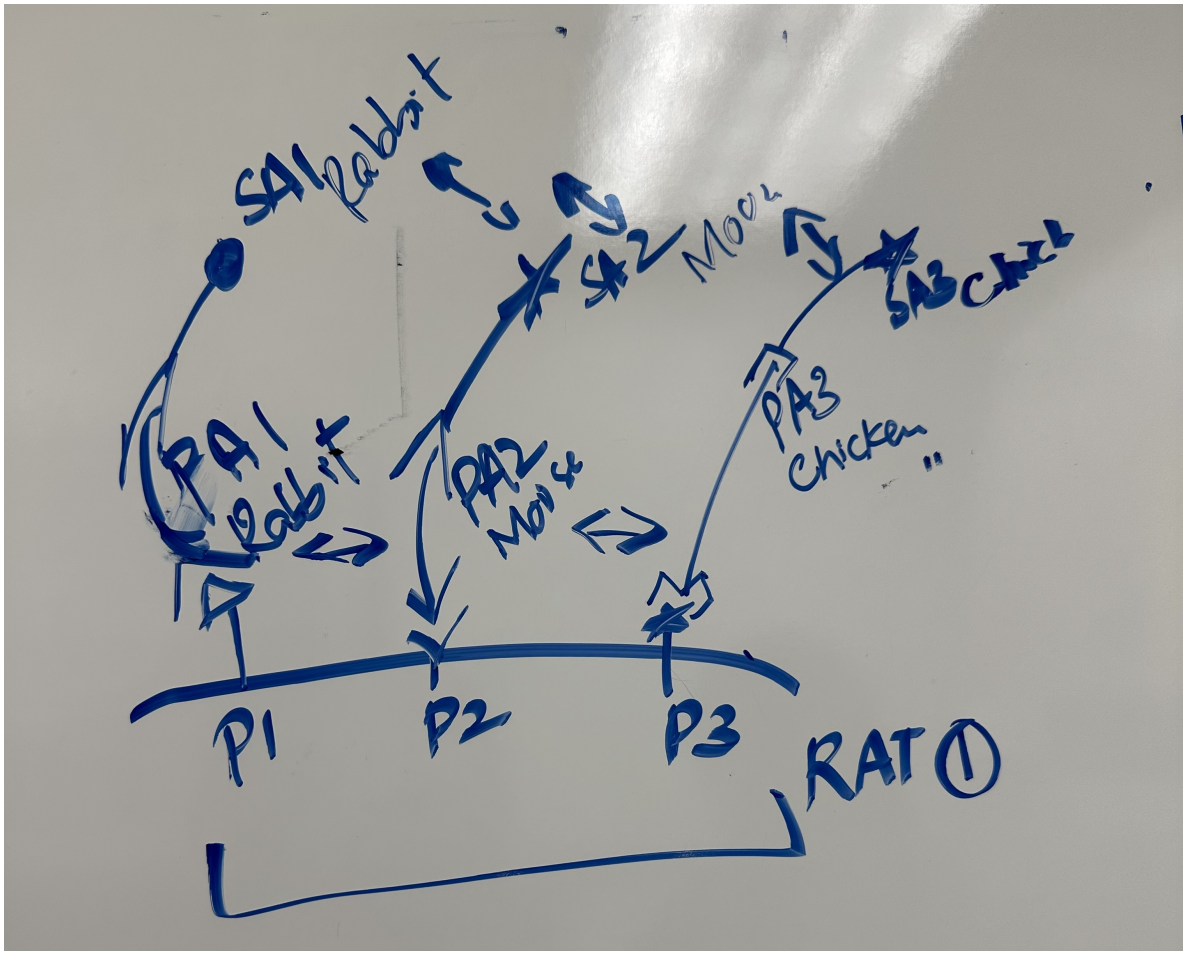
- Questions:
  - Why different antibodies?
  - Refer to Dr. Garcia's points
  - Get a sense of the calendar for the next months
- Followed pt. 2 of the immunocytochemistry procedure:

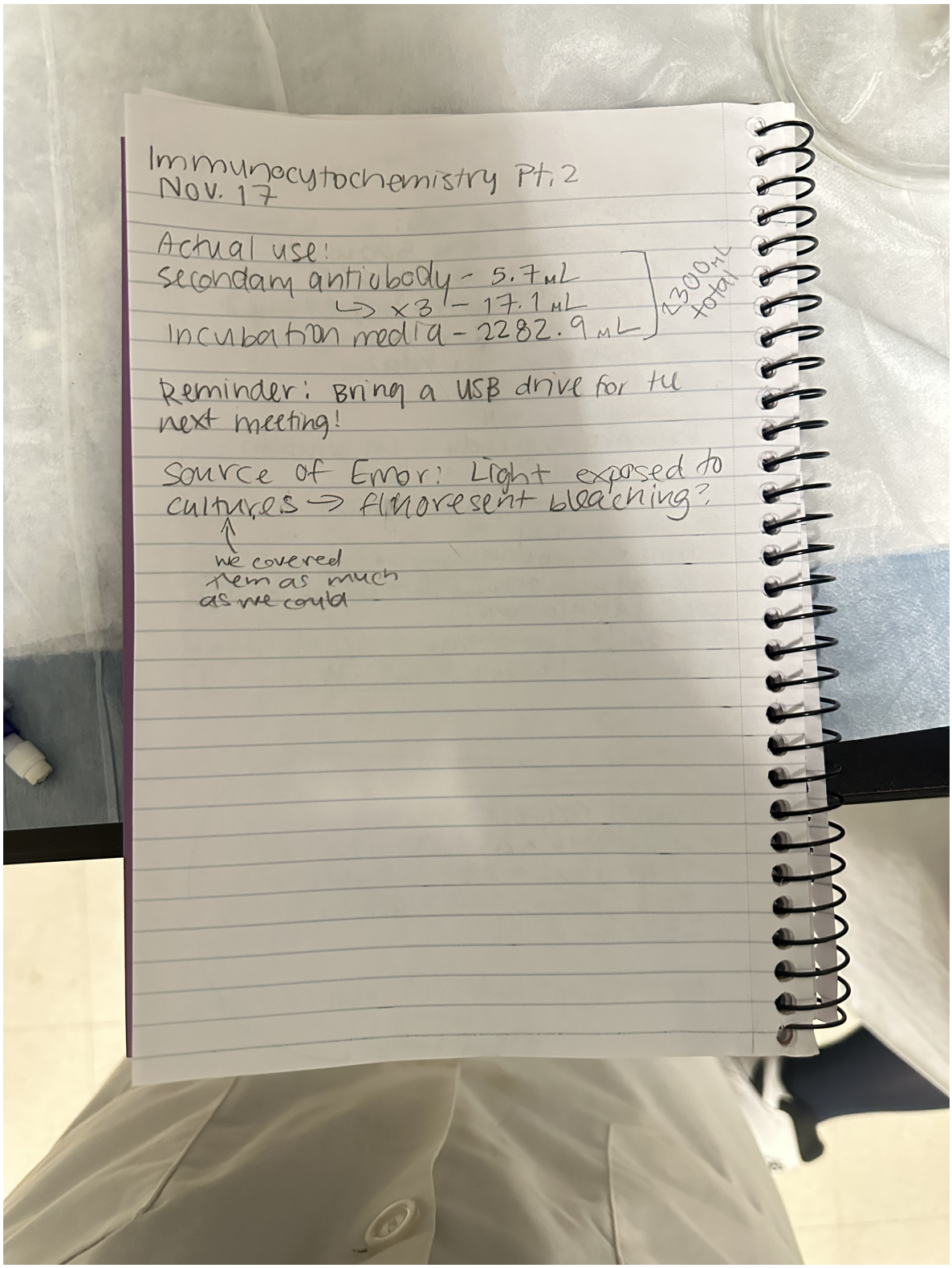
9) Wash 2 times for 10 min with 1x PBS (1 mL)
10) Prepare secondary antibody solution 1:200 diluted in IM
11) Add 100 μL of secondary antibody solution per dish

- **Keep like this for 2 hours in dark (RT) *In the dark***

12) Wash 2 times for 15 minutes in PBS (1 mL)
13) Rinse briefly with dH_2O (1 mL) and let air dry (Leave lid half on) for 5-10 minutes while kept in the dark.
14) Mount
- Take out water
- Put 3 drops of Mowiol into well
- Put on coverslip (1 more drop for coverslip) – Let coverslip drop to the well parallel to it to avoid bubble formation.
- Put in fridge and image next day

- Notes:


SA1 Rabbit
SA2 Mouse
SA3 Chicken
PA1 Rabbit
PA2 Mouse
PA3 Chicken
P1
P2
P3
RAT ①



▼ ***November 21 (Class)***

ASP Work Class

- Next logbook due: Nov. 30th

    - Make a plan for every class in December
- Dr. Garcia talks about CYSF portal
    - Make sure you have logged in (we have)
    - Fill out basic project info (we have)
    - Ethics (need mentor's name, email, ethics code, department of u of c)
- Planned each day of the calendar for December and updated the November calendar
- Note: At the end of the introduction section (lit review) we must add goals/research questions

▼ ***November 23 (Class)***

ASP Research Proposal Presentations (Amy, Ashank + Joel, Tiffany)

- **Amy's Presentation:**
    - This is a study
    - 12-17, 18-24 very high risk groups
    - Males commit more violent crimes
    - psychopathy (18-40% of all crime offenders)
    - Neurobiological traits between these groups
    - older 25+
    - Limited data and info
    - Research for psychopaths are very limited
    - Zz Q: What is a Z test and how does it work?
        - it is a statistical data analysis.
- **Ashank + Joel's Presentation:**
    - Auto-immune response: body things bacteria is its own cell
    - Through metabolic pathways, metabolites are created which could lead to diabetes.
    - Type 1 Diabetes:
        - people with type 1 diabetes can't regulate their blood sugar levels bc of an autoimmune reaction
            - Does the body think that their own antibodies are bad?
    - DIABIMMUE Study
        - feces into robogut and sequences the genes, bacteria
        - 2 groups of mice that were implanted into
        - monitored
        - sequenced genomes after
    - Python script to take data from output file
        - identify mimotopes
        - identify metabolic pathways
    - greater understanding of mimotopes.
    - production of vaccines and treatments for type 1 diabetes.
    - children from Latin descent are more likely to get diabetes.
    - Tosin Q: How will you analyze the pathways (significance tests?)?

        - The output file will allow you to see which sample correlates to which mimotope and that's how it will be analyzed
- **Tiffany's Presentation:**
    - Trauma from a first responder job
    - Depression, Anxiety, PTSD
    - Adrennalin and noradrenallin
    - Stress on the hippocampus
    - long-term exposure causes dendrite shrinkage
    - Amidgila does more than it needs to
    - Long-term stress causes shrinkage of the pre-frontal cortex
    - Loss of fear memory, not afraid to do reckless things
    - Therapy, EMDR, SNRIs
    - Zz Q: When they take these anti-depressants is that for the rest of their life, does it cause harm to the brain, is it actually fixing anything or just a temporary relief?
        - They will not, they use it until the issue is mitigated, in conjunction with behavioural therapy, it is a temporary relief
    - Tosin Q: How will you identify what is a large disparity or not?
        - She will just look at what is available vs. what is online? Not very well explained tbf
- Note from Dr. Garcia: Everyone needs a punchline. If you don't have a quick explanation of your project at the beginning of your presentation, that is not good. If you can't explain you project in a couple sentences, you don't know the project well enough.

▼ ***November 27 (Class)***

ASP Work Class

- December 15th: Intro Section of the final paper
- Calendar for December:
    - Lots of Reading
    - Deep understanding of theoretical aspects.
- MAIN FOCUS:
    - Data collection
    - Deeper understanding of methodology (how it answers the research question)
- Dec/Jan:
    - Winter break (conversation)
    - Midterm period (12th-22nd)
    - Where can we fit in ASP work?
- We should have 1 meeting/week
- We should have 1 email/week
- Time will be taken off for midterms
- February: Posters and presentations
- School Science Fair: March 4th
- Sent an email to mentors to discuss the future of the project

    - When can we meet to start data analysis?
    - DA needs to be done by end of Jan/beg of Feb
    - We are available during winter break (Dec 21-Jan 7)
    - Jan 12-22 We will not be available due to midterm exams
- Sent an email on CYSF portal about delay on ethics approval
- Read over RP comments and made the “small” edits
    - Things that require rewriting will be done next class

▼ ***November 29 (Class + Check-in)***

ASP Work Class

- Dr. Garcia spoke about the presentation in February
    - Presentation days are strict
    - Trifold or flat poster
    - make a plan for the poster
    - Start thinking about how we will prepare the presentation since ZZ will be gone for a while.
    - Make a plan for how we will prepare the flow of our poster
    - Make sure you understand your project really well
- The CYSF people responded to our email: “We're not ignoring you. Because of the Ketamine and CBD oil, approval had to go to the national level and we're waiting for an answer from them.”
- Continued editing the RP intro (literature review)

Dr. Garcia Check-in

- EMAIL!!
- in depth understanding of why each dye binds to each section
- live/dead assay/ immunocytochemistry
- ask about concentrations
    - dose increase/decrease?
- conclusions and future directions
- how can raw data answer your questions
- adjust timing for background (decrease)
- methodology is important, but results takes the most time (major takeaway)
- full circle ending
- ask mentors: can someone be under marijuana and ketamine in a clinical situation?
- emphasize importance
- cognitive function relatin to celldeath, synaptic connectivity, and growth
- presentation should come naturally (know it deeply)
- insist on weekly meetings (minimum) for data analysis (they give us tasks, they give us results)
- make a long-range plan and send another email w/ dates
- ask if lily can help with data analysis

- bc zz will be gone early, try to be done (mostly) everything before she leaves
- Questions for Dr. Garcia/Topics:
  - Ask Dr. Garcia about the first paragraph of the intro (should we turn that into the abstract or should we leave it as the intro?)
    - Don’t worry about that for now
  - Ask to switch our presentation day to the last day because ZZ will be gone
    - We discussed with Tiffany and she agreed to switch with us, Zz will email and remind Dr. Garcia
  - We emailed our mentors, still no reply
    - Email again!

▼ December

▼ *December 1 (Class)*

ASP Work Class

- Sent an email to Dr. Garcia stating that we will be presenting on March 1st instead of February 28 since Tiffany has agreed to switch with us.
- We have about 6 weeks left until the presentations
  - Each week - filled with tasks
    - Logbook
    - Reading A/B/C
    - Literature (Intro)
    - Data Collection
- Made a general plan going forward until project oral presentation:
  - Dec-Jan: Data analysis, results, conclusion
    - Dec 21-Jan 7 is Winter Break (Tosin & Zainab are staying in Calgary)
    - Jan 12-22 are Midterm Exams
    - Zainab K. travelling (date?)
  - Early to mid-Feb: Poster planning and presentation practice
    - Feb 15-19 Family Day Long Weekend (Zainab is gone from Feb 14-25)
  - March 1st: Oral presentation
- Sent an email to Zainab K.:
  - **Summary:** Tosin and I plan to complete quantification and data analysis before the Winter Break, dedicating that time to analyzing our project data. The timeline includes data-focused work in December/January, Winter Break in Calgary from December 21st to January 7th, Midterm Exams from January 12th to January 22nd, Zainab K.'s travel (date TBD), poster planning and presentation practice in early to mid-February, Zainab's absence from February 14-25 for the Family Day Long Weekend, and concluding with the oral presentation on March 1st. Specific travel dates for Zainab are pending confirmation. Any questions or additional date details can be addressed.
- Continued editing the RP intro (literature review)

▼ *December 5 (Class)*

ASP Work Class

- Dr. Garcia discussed the logbook marks (she is being generous this month, however next month she will start failing people who are not meeting logbook requirements; LOGBOOKS MUST BE DETAILED; experimental

procedures and data collection sections should be filled in when that information is available; tasks should be more specific)

- Emailed Zainab Khan to attempt to meet on Friday after school
  - Availability for Imaging Session:
    - Thursday: 11:50 am to 1:30 pm
    - Friday: After 4 pm
    - Every weekday: After 4 pm
  - Please confirm a convenient time for the session
  - Looking forward to your response
- CYSF got back to us:
  - "Hi Tosin and Zainab: We've received a ruling from YSC on your project and I would like to send you the files. I can't attached anything to these messages within this platform so I would appreciate it if one (or both) would send me your email(s). Leslie" (Dec 1)
  - "Tosin and Zainab: The essence of the ruling seems to be that, should you go to the Canada-Wide, you be able to prove that the mice weren't killed specifically for your project. To that effect, I have a form you need to get filled out, hence my need for an email. Leslie" (Dec 3)
- We replied:
  - "Hello, So sorry for the late reply. This is Tosin's email: babayejutosin@gmail.com. This Zainab's email: zainabsoliman24@gmail.com." (Dec 5)
- **Completed final revisions for the intro section of the final paper**
  - Made all edits that Dr. Garcia suggested
  - Added the research questions/goals at the end
  - Sent Dr. Garcia an email to approve the lit. review/intro

  https://docs.google.com/document/d/1qVqHifjjpGAI148GL7p4vZH7PifprRukvqoxjRGmBR8/edit?usp=sharing

- Dr. Garcia wants a **detailed** plan moving forward ready for the next biweekly checks
  - Talk to mentors about this

▼ ***December 7 (Class)***

ASP Work Class

- Dr. Garcia spoke
  - Presentations in class will be March 1, Feb 26, Feb 28
  - March 4th, Science Fair goal
  - MAKE SURE DETAILED PLANS ARE MADE
  - If you will not finish in time, find out what is non-negotiable so that something can be presented
- Sent an email to mentors about ethics approval questions:
  - We are seeking approval for our project at the Calgary Youth Science Fair
  - We've received a list of questions that must be addressed for the ethics review process

    - We're reaching out to the mentors for collaboration and support in tackling these questions to ensure the ethical integrity of our project
- Sent an email to mentors about meeting for data analysis
    - Can we do it during the winter break if possible?, if not, we will have to miss some class
- The rest of class time was used as a work/study period for other subjects (Dr. Garcia approved)

▼ ***December 11 (Class + Check-in)***

ASP Work Class + Check-in

- Went over month outline with Dr. Garcia
- Have a planning meeting over zoom to figure out plan and figure out what we will do
    - Know which days we are going into the lab for imaging/data collection
    - Know the tasks
        - experimental work that has to be done
            - how long, where (in the lab/at home), and when
        - what comes next after the images of the raw data
        - can we do the data analysis at home?
        - get a plan of what else we have to do that hasn't been done yet.
        - bring the steps to the meeting for each task
            - how long
            - where
            - when
        - any data collection/analysis we dont know of?
        - what are the new steps for data analysis that we need to do (by when, how long, where)
        - put all of this into a calendar divided into each class and times we can go to the lab
        - re-visit this before the break with Dr. Garcia
        - must give a lot of time to poster presewntation and oral presentation (get their advice for the layout of the project)
        - can have some images of raw data but must descrive it
        - get presentation done on top of poster
        - meet with DR. Garcia on December 19th
    - Zoom meeting:
        - timeline
        - CYSF questions
        - imaging
        - images to be analyzed (immunocytochemistry)
        - waiting to image brain cultures
- Emailed Zainab for Zoom meeting based on what was discusses w/ Dr. Garcia
    - Request for a Zoom call to discuss upcoming steps for the project
    - Suggested meeting dates and times:
        - Wednesday, December 13th: 9:55 am - 12:10 am

        - Thursday, December 14th: 11:40 am - 12:10 am
        - Friday, December 15th: 11:40 am - 1:45 pm
    - Filled out “Experimental Procedures” section of logbook (added all the info of the experimental work we have done so far)

▼ ***December 13 (Class)***

ASP Work Class

- CYSF: We have received Ethics approval; we now have to fill out 2B form on portal and a physical copy
- Filled out 2B form (nothing was saved after 45 minutes of work, will redo again next class)
- Received feedback from Dr. Garcia on introduction section of paper, made all edits that she suggested (mostly consisted of removing irrelevant information or adding extra details)
    - Submitted it on google classroom

https://docs.google.com/document/d/1qVqHifjjpGAI148GL7p4vZH7PifprRukvqoxjRGmBR8/edit?usp=sharing

▼ ***December 14 (Zoom Meeting)***

- Ask Zainab K./Mentors: How will the Participants be recruited? Give the criteria by which participants are (a) included and (b) excluded from the study. Describe the source of the participants and the manner in which they will be recruited. (We need it to be clear that the rats had a greater purpose in the lab and we did not just kill them for our purposes)
- Ask for: Phone # of Dr. Syed
- (Check Dr. Garcia's check-in notes and general plan in quick notes and calendar notes)
- Refer to calendar for the rough notes of the meeting

▼ ***December 15 (Class)***

ASP Work Class

- Sent an email to reschedule meeting for imaging
- Sent an email to Zainab k. to remind her to update the timeline w/ tasks before Tuesday as that is when we will be showing Dr. Garcia
- CYSF: We have ethics approval and the Significant Risk 2B form is not needed to be completed unless we are selected for the Canada Wide Science fair. In that case, we will complete it then.
- Watched various videos on cell cultures/live dead assay to try to further understand methodology:
    - https://www.youtube.com/watch?v=RpDke-Sadzo
    - https://www.youtube.com/watch?v=22uS5Ls44MU&t=37s
    - https://www.youtube.com/watch?v=lJmDYfTaAy4&t=43s
    - https://www.youtube.com/watch?v=z5CtZ73sMMc

▼ ***December 19 (Class)***

ASP Work Class + Check-in

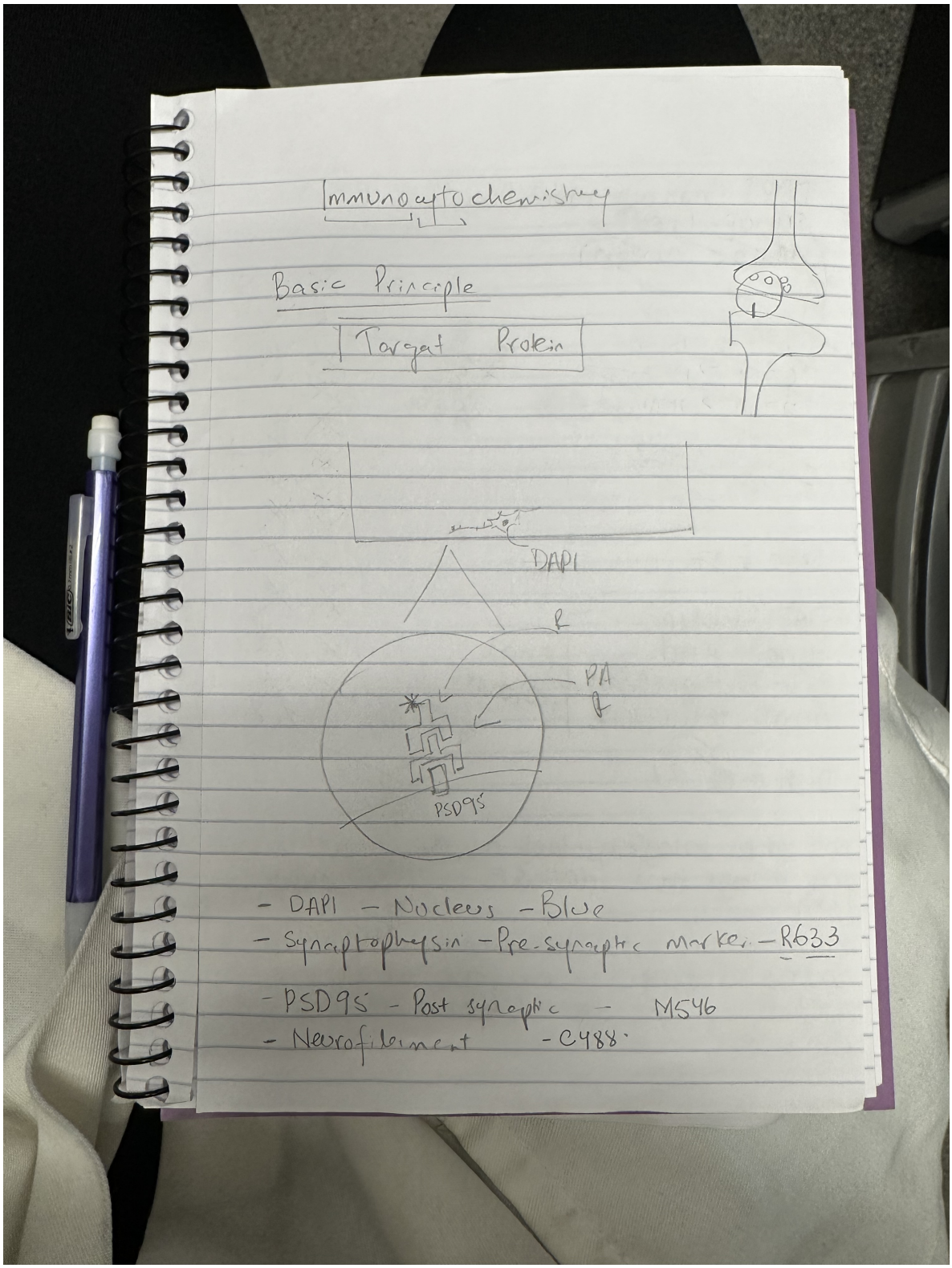
- Next assignment is the methodology section (can use rp one as a baseline, still edit it, ours is too detailed)
- Showed Dr. Garcia our in-depth monthly outline (in the “Quick Notes” section of logbook)
    - Told her about zainab k. methodology presentation

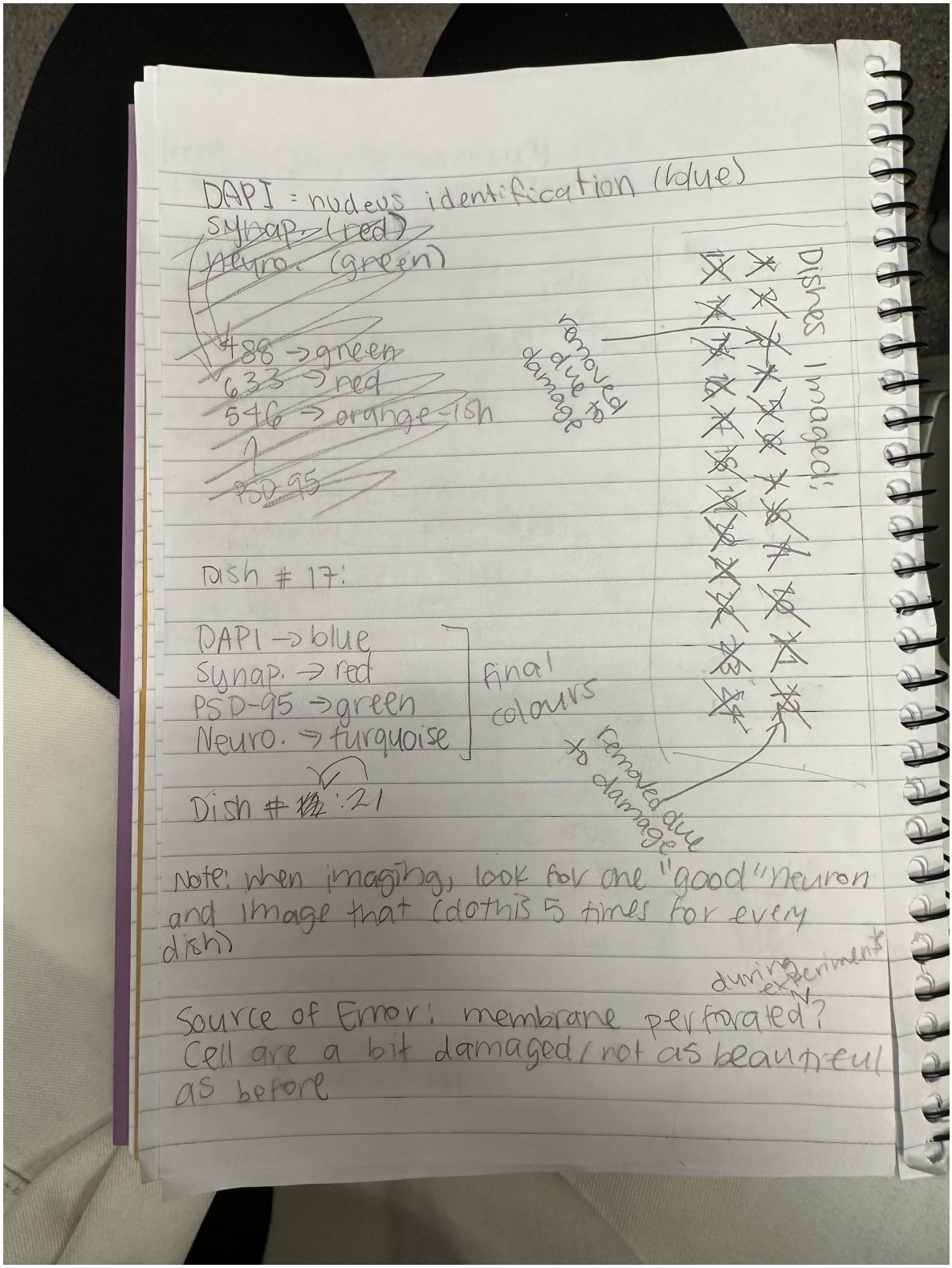
- Cut back on background, put more time into methods
- Try to add more graphics (you can use this in your poster/other presentations)
  - Use biorender to help with this (search up templates)
- What is the best way of explaining the results? Graph, diagram, tables?
- How much analysis will we get done in the meetings? The final analysis (diagrams), when will they be finished?
- Figure out how to put plan into practice, put it into the calendar, divide the tasks
- Take detailed notes of meetings to gain deep understanding
  - Get notes in own words but get technical language
- We can set up a time to present with the poster (more informal than the powerpoint oral presentation)
- Be very flexible during the presentation (don't be attached to a script)
- Watched various videos on cell cultures/live dead assay to try to further understand methodology:
  - https://www.youtube.com/watch?v=ABWzA9kjDbY
  - https://www.youtube.com/watch?v=-TVs_mVGuh4&t=109s
  - https://www.youtube.com/watch?v=3pdMQNJz9TQ
  - https://www.youtube.com/watch?v=srfCX9XvXbc&pp=ygUSY2FsY2VpbiBhbSBhbmQgZG5h

▼ ***December 22 (Lab Visit)***

ASP Lab Visit

- We imaged all 22 dishes (for the immunocytochemistry)
- We previously did INDIRECT immunocytochemistry (this is because DIRECT immunocytochemistry limits the number of proteins that we can target)
- "Immuno" antibodies, "cyto" cells
- We will meet again to collect all the images on the usb
- We will schedule a zoom meeting to go over all the stats/analysis
- Notes:


Immunocytochemistry
Basic Principle
Target Protein
DAPI
PA
PSD95
- DAPI – Nucleus – Blue
- Synaptophysin – Pre-synaptic marker – R633
- PSD95 – Post synaptic – M546
- Neurofilament – C488.



▼ ***December 29 (Lab Visit)***

ASP Lab Visit

- We copied all the images onto the usb
- We were shown by Zainab K. how to do live/dead assay analysis ONLY
- Zainab has shared documents with us with step by step instructions
- We will do the rest of the analysis after this, but this is all for now
- We may only be doing cell viability and synaptic connectivity, however we may be able to use the synaptic connectivity analysis to measure neurite growth that way
- We will finish the the cell viability analysis before the end of winter break (Tosin/Zz will be splitting the analysis work in half)
- Zz tried doing the analysis on her computer to see if it worked, it did!
- We will contact Zainab k. via email or zoom meeting if we have any questions
- We are using single blind analysis (we don't know which dish was treated with what) to ensure that there is so bias when running the analysis
- All data is logged in an excel spreadsheet which will then be inputted into a software that creates our graphs/does the statistical analysis
- Things to get started on: analysis, methodology presentation/our presentation, poster design

▼ January

▼ *January 8 (Class/Midterm Prep Period)*

ASP Work Class

- Worked on Methodology presentation for Zainab K. (so that she can see how well we know it/help us fill in any gaps)
  - Made a slideshow with a flowchart for the methodology
    - We still need to attach more graphics and images
    - Link: https://docs.google.com/presentation/d/1T49F4eb4mXofNmh25UH68GldyGb3ZC1A2inq3eMqGw0/edit?usp=sharing
  - Made a google doc explaining the entire methodology of our project (in detail) as we know it, and writing down any question that we need clarified before our final presentation
    - We still need to add more detail in the specifics of the function of the antibodies/proteins as well as the viability staining/dyes
    - Link: https://docs.google.com/document/d/1XAGxMKWclnet_CazppwSTlpkf4VFjJmYfGZI7SLEy-M/edit?usp=sharing

▼ *January 10 (Class + Zoom Meeting)*

ASP Work Class + Zoom Meeting w/ Fahad and Zainab K.

- Continued working on methodology google doc
  - Link: https://docs.google.com/document/d/1XAGxMKWclnet_CazppwSTlpkf4VFjJmYfGZI7SLEy-M/edit?usp=sharing
- Meeting was intended to discuss our next steps in the project/address any questions about methodology as we had finished the analysis for the cell viability and were ready to analyze the immunocytochemistry images
- During the meeting, it was discovered that the data we collected was not very accurate as the program being used was not adjusted for the brightness level of the images
- Fahad was able to adjust the code for the macros on the program, that we were using to more accurately capture the neuron cells in the images

- Because of this we cannot move on to the next steps of analysis yet and must redo the cell viability analysis
- During the meeting we also discovered that because our experiment is part of a greater experiment that Fahad (grad student, mentor) is doing involving seizure induction, some of our dishes underwent seizure induction which could affect our data
    - We may need to include this aspect in our project even though this was not originally planned
- The images for Dish 1 have not yet been processed as they are still in the .czi format, we have to download the "zen blue" software to convert them to .tif files to be analyzed
    - Ask Fahad/Zainab for a link for downloading this, could not find it online

▼ ***January 11 (Meeting w/ Dr. Garcia)***

Impromptu Meeting w/ Dr. Garcia

- Met with Dr. Garcia to discuss seizure induction addition to the project (how will it work?)
- In order to avoid having to learn all about seizures/epilepsy and their effects on the brain in conjunction with CBD and ketamine, we will likely either:
    - Use all the no Mg/seizure induction data (but mention in the results section that seizure induction could have affected results)
    - Use all the Mg/no seizure induction data (but mention in the results section that seizure induction could have affected results for the control, which had seizure induction)
    - We cannot compare both sets of data because then we would need to add seizures/epilepsy into our project which affects the introduction/background, hypothesis, research questions, goals, etc. (work that took a while and that we have finished a while ago)
- We could potentially make another set of control dishes to use that did not have seizure induction, or possibly use control data that the lab has previously produced as we need to true baseline for our control group so that effects can be properly compared
- Before making any further decisions, we will meet with our mentors to discuss how we will attack this
- We are planning to redo our cell viability analysis after midterm exams when we are less busy

▼ ***January 18 (Zoom Meeting)***

Zoom Meeting w/ Fahad and Zainab K.

- We discussed the questions that we had about our methodology to clear everything up
- The last meeting with Zainab as she leaves tomorrow
- Concluded that we will use all seizure-induced data as it will be a common control among all dishes.
- Fahad mentioned that we should add seizure induction mechanisms into our intro
- We will go to the lab next week to process the ICC images.
    - Link: https://docs.google.com/document/d/1XAGxMKWclnet_CazppwSTlpkf4VFjJmYfGZI7SLEy-M/edit?usp=sharing
- Asked Questions about the project/general questions
- Ask about Dish 1/Dish 3 situation, Dish 2 results were diff at home then at school (address the table: only dish 12 is listed to be removed but dish 3 was, Mg (why is it added?), spreadsheet says **no PBS wash**)
    - 1 is in file 9 just not the file type we need it in.
- The cell cultures were left in an incubator for a week to allow the cells to grow and connect (how is this done in-vitro?)
    - Mg/no magnesium is important for epilepsy, seizure, uncontrolled activity for brain cells, excitatatory neurons vs inhibitory neurons. Glutamine is released, minds to receptors, from pre-synap neuron, attatched

and binds to post-synpatic neuron, ampa or NMDA receptor. NMDA receptors open when glutamates bind and allow neurotransmission, influx of ions, calcium sodium, NMDA wont open right when glutamate is released only when there is sufficient de-polarization.

- What is the media composed of and its exact function?
  - Composition is on the protocol.
- How long did we wait before exposing the cell cultures and why?
- How does putting the pups in ice anesthesize them?
  - Blood flow slows down
  - The animal goes to sleep.
  - Sleeping don't
- Why did we use P2 rats?
  - Use 0-2 , get as close to birth
  - Ideally, p0
  - Less robust the synaptic connections are
  - More malleable
  - Can regenerate
- What does the digestion solution do to the hippocampus?
  - Has papaine
  - EDTA
  - An enzyme a protiast
  - Breaks the peptide bonds
  - Breaks away protein bonds
  - Helps for the next step of
- After the substance exposure, why do we incubate the dishes for 1 hour, how does that reflect how the drug is used in real life?
  - No answer D10 could be a young adult human
- What does the poly-d-lysine do for cell plating?
  - Glass was used, poly-d-lysine and laminen, lam is a glue that sticks the neurons down and holds them down, polydlysine is positive and coats and adheres to the glass, the neurons and negative, the laminen in there to act as a glue.
  - ED 50, dose-response tests, equivalent for clinic
- I know that we are imitating medical exposure concentrations but which dieases are we directly imitating because I know that different concentrations are given for different dieaseses.
- On the document it says salt solution for 10 minutes, what is that and what does it do?
  - Tosin explain current knowledge of live/dead assay and immunohistochemistry and then fill in the gaps of confusion

▼ ***January 24 (Class + Check-in)***

ASP Work Class + Check-in

- Dr. Garcia's Announcements
  - There has been an increased use of AI in the school

        - Reminder to not use AI in this course, please hand in the introduction section of the final paper to turnitin
    - Important Dates:
        - Jan 31st - Dec/Jan Logbooks
        - Feb 12st - Procedures Section Deadline
        - March 1st - Marks lock @ 9AM (No Science Fair in the Term 2 Report Card)
    - Procedure's section should be brief and succinct
        - Don't need to *deeply explain* your methods, because your audience should know them
        - Must be in perfect past-tense
        - What was done/How was done (not why!)
        - Include subsections
        - Include ethics
        - Citing a kit/equipment/software/programs (even Excel): (Name of Manufacturer, City, Country)
    - Results (or Methodology) should be the core of the methodology (should take up about 5 mins/half of the project)
    - Intro should take 2-3 mins MAX, should show why your project is important
- Both of us worked on the analysis of the cell viability (as it had to be redone due to the tech not being correct the first time, explained in depth in previous entries)
    - We ran the ImageJ program on our computers to analyze the images, which gave us the number of live and dead cells, we put this data into Excel to store it and get the cell viability percentage (we finished about half, planning to finish later today)
- Dr. Garcia meeting:
    - Gave her an update on what has occurred since the last meeting
    - Seizure induction should not (have to) affect the intro, hypothesis, research questions, etc. just the procedure and the results/conclusion (however if it ends up making a huge part of the project then a paragraph should be added intro?)
    - The analysis for cell viability and ICC should be done by the weekend, leaving February to prepare for the project (writing, graphics, poster design, presenting prep)

▼ ***January 25 (Lab Visit)***

ASP Lab Visit

- Questions/Things to bring up:
    - export ICC Zeiss
    - How would we cite the macro in our final paper? Do we need to?
    - Added section for the intro (just NMDA receptors? Is it necessary b/c Dr G. says no?)
    - Links do not work for dish 1 (do it there?)
    - How would we explain how this relates to cultures that haven't been seizure-inducted
    - Teach us how to do ICC analysis (how long will it take, can we include neurofilament growth into this?)
    - Check out the outliers of the cell viability data
- Meeting notes:
    - Fahad looked over our cell viability data and it seems to look good

- There are some outliers in the data so he suggested that for those images, one of us should just count the neurons manually
- We extracted the data/images for dish 1 so that they can be analyzed
- We went through all the images for each group that we are analyzing to find one image to represent the group
  - We did this as our images we not good enough for quantitative analysis
  - Using these images we can do qualitative analysis for the growth and synaptic connectivity
  - If we want, we can retake the images later on so that we can get quantitative data but it cannot be done now
- Fahad says that the info about the seizures cannot only be mentioned in the results section and are necessary in the intro because you cannot just add new information at that point (discuss with Dr. Garcia)
  - Talk about seizures, epilepsy, and the nmda receptors
- Macros do not need to be cited in our procedure as we made them
- Fahad talked about how neurons do not just make connections at random, some neurons emit signals that other neurons are “attracted” to and which is what facilitates the proper connections of neurons
  - Substances like CBD and Ketamine can disrupt these processes and signals so neurons connect to others at random that they are not meant to connect to
    - Through its interaction with the CB1 receptors, CBD may also increase the activity of inhibitory neurons and decrease the activity of excitatory neurons
  - This MAY increase synaptic connectivity but not in positive way
  - This also decreases the efficiency of the neurons in the brain
  - This MAY contribute to the cell death of the neurons
- Some notes on data/images taken:

  Note Jan 25, 2025.pdf

▼ ***January 26 (Class)***

ASP Work Class

- cell viability updating data and adding dish 1 data
- Analyze dish 1 data and added it to the document.
- added cell viability procedure to the procedure document.

  https://docs.google.com/document/d/1XAGxMKWclnet_CazppwSTlpkf4VFjJmYfGZl7SLEy-M/edit?usp=sharing

- Continued working on the document that has each depth/explanation of our procedure in depth for learning purposes.
- We cannot present our methodology presentation to Zainab as she had left already.
- Read the requirements/expectations for the procedural section and we will begin working on it next class.
- Went through the Excel spreadsheet with our raw data and went through the highlighted boxes (outliers) and eliminated extreme outliers or images that are just too blurry to be used.

    - These outliers have been removed
- Excel/ImageJ needs to be cited
    - ImageJ definitely needs to be cited
    - Excel most likely needs to be cited (only add if it is part of our procedure which it kinda is but we used it)
- Fahad said the macro does not need to be cited as we wrote it together
- general lab stuff doesn't need to be in the procedure (media composition)
- use staples for the project if using a layout poster
- Mayo Clinic citation must be changed
    - this is not a reliable/valid source
- poster; show how immunocytochemistry works
    - one of the important quantification steps that should be explained.
- include Fahad source of error PBS wash (in the future we would..)
    - Fahad made an error on dish 12

▼ ***January 30 (Class)***

ASP Work Class

- Updated background research sections
- Updated experimental procedures
- Updated data collection and results
- Updated schedule and tasks
    - Did February tasks
    - Not accurate as we have to discuss it with mentors
- Updated daily notes
- Checked on the CYSF site
    - no new updates but we looked at the designs of previous projects
- Made measurements for the trifold
    - 152cm/5ft/60in (height)
      80cm/30in (left/right panels)
      82cm/32.5in (centre panel horizontally)
- Planned out what should/must be contained in the poster design layout
    - the centre should highlight the results/any data tables etc.
    - the left side could have background info
    - the right side could be the conclusion/acknowledgements etc.

    https://docs.google.com/drawings/d/1dV3_FCtk4dLoF4R2aczKBSFOWi1BXRvdmyToV21Bahw/edit?usp=sharing

- Transferred the methodology from our intro into the new document.
    - Spend the next class working on the procedure section and if done, send a draft to Dr. Garcia by the end of class.

▼ February

▼ *February 1 (Class + Zoom Meeting)*

ASP Work Class

- Monday, March 4th (Science Fair - Missing AM Classes)
  - Make sure to email teachers
  - 8:15AM -8:45AM (Poster set up)
  - 9:00AM (Presenting and judging)
    - 5 Judges, 30 minutes each
  - 11:30AM (Done)
  - Results come out Tues/Wed
- Did the Science Fair google classroom assignments
- Worked on poster design
  - Overal outline of the layout, colours, and where each section would go was picked
  - Title w/ symbols and names are done too
  - Link: https://docs.google.com/drawings/d/1dV3_FCtk4dLoF4R2aczKBSFOWi1BXRvdmyToV21Bahw/edit?usp=sharing
- Looked at different printing companies for printing (staples, little rock printing, digital post printing)
  - Contacted Lily for recommendations (little rock printing)
- Planned what we will do in today's zoom meeting (questions)

Zoom Meeting w/ Fahad

- Questions:
  - Salt solution procedure
    - Still do not have this - contact fahad through email
  - How will we qualitatively analyze neurite growth? How can we do this?
    - If we see a significant difference, this can be commented on by descriptions
  - What should the qualitative observations/results look like?
    - Just describe what you see
- Learned how to use Graph Prism and got the final graph that we will use
- GraphPrism Login:
  - Email: zainabsoliman24@gmail.com
  - password: zztosin2024

▼ *February 5 (Class + Check-in)*

ASP Work Class

- Dr. Garcia spoke about logbooks/science fair
  - She likes the notes and neat things but also photos and raw data
  - Arrows, analyzing, links to a spreadsheet
  - When we link a procedure paper or link in general, make sure Dr. Garcia can open it

- Re-tract portal note
- The portals will be reviewed by the judges
- 10-15 minutes of presentations
    - **Background**
        - Dont talk about what the brain is, no basics
        - maybe a sentence or two of general info
        - if methods and results don't take as much time, then more time can be put into background
        - dont mention common knowledge
        - go straight into project
    - **Research Questions/Goals (Mention variables)/Hypothesis**
        - before getting into methods: what do we want to do?
    - **Methods**
        - graphics/flow chart
    - **Results**
        - center of the poster/presentation (main focus)
        - graphs tables
        - for us: synapse images, live/dead
    - **Analysis**
        - Explain results and use literature
    - **Conclusions**
        - Answer research questions
        - Did results support the hypothesis
        - Summary of what was achieved
        - Bullet points
        - Questions that are not answered/not clear/no difference
    - **Future directions**
        - New questions/experiments/designs/address ambiguous things
        - What does the literature say
        - What is next? what we would do different in the future
        - What was wrong (how to fix)
        - What's next
        - Be critical and nitpicky with the procedure, how would we design a new experiment
        - Important: good critical analysis of results and a way of testing
    - **Significance**
        - What have we contributed to the field of study
        - Important for a new avenue of research
        - Between analysis and future directions
- General notes:

        - Clearly labelled graph: explain it heavily
        - Loud, animated, make eye contact, be confident, Q-cards (try to avoid)
        - The flow of the project should be smooth
        - Refer to the judging scoring sheet that Dr. Garcia posted
        - For the methodology, understand the methods in and out, the whys of each step
    - Posters only:
        - Acknowledgements
            - Funding (ask eg. NSERC, CHIS, heart and stroke foundation))
            - UofC, access to lab equipment and expertise, mentors
        - References: small section (summary of the most important ones)
        - For the poster, make effort to highlight certain sections
- Check-in with Dr. Garcia
    - Tell Dr. Garcia that we only have 2 research question now, show the graph
        - Images for synapse are not clear enough to quantify
    - For results we only have 1 graph, synapse images per 4 conditions, live/dead images per 4 conditions?
    - Should we mention sources of error?
    - Say what is wrong it the images
        - How would you do it if you had the correct ones
    - Clarify with fahad why the graphs look the way they do, 2 way anova, why the graphs cannot be more clearly labeled (is there a way to make it clearer?)
        - Understand why the stats are the way they are
    - Continue with image analysis (control, ket, double, come up with a solid solution/ explaination) select diff images that are good then make final selection at the end
    - Work on understanding, once presenting, do not mention what mentor said
        - Keep asking them questions
- Sent an email to Fahad: “Tosin and I have a few more questions regarding the graphs and making it more clear for a general audience to understand. We have also selected new images for the synapse photos and would like to verify them with you. When are you available for a Zoom meeting?”
- Worked on procedure section of paper
    - Completed all the methods up to cell plating, just need to finish cell treatment, cell viability, ICC, and statistical analysis
    - Document link: https://docs.google.com/document/d/1BlfzZ2WkrblYYKXVE0Q8czXcN0wT-XlJukCsyWYtlDg/edit?usp=sharing

▼ ***February 7 (Class)***

ASP Work Class

- Reminder: If you are moving on to the CYSF, portal must be completed beforehand
    - Portal closes: March 15th @ 9AM
- Emailed Fahad to confirm zoom meeting for tomorrow
- Worked on procedure section of paper
    - Finished cell treatment, cell viability, ICC, and statistical analysis (done rough draft of methods paper)

    - Goal is to send to Dr. Garcia by this weekend
    - Document link: https://docs.google.com/document/d/1BlfzZ2WkrblYYKXVE0Q8czXcN0wT-XlJukCsyWYtlDg/edit?usp=sharing
- Prepared questions to ask Fahad in the next zoom meeting:
    - Try to understand 2 way anova
        - Still need to do further research/watch videos on this
    - How can the graph be fixed
        - Graph can be edited through the GraphPrism program
    - We have images that include glia: do we have to come in and re-image (only the groups we are analyzing)
        - No, we will say that these are images of our “cell cultures” not just neurons, and we are planning to reimage in the future to get quantitative results

▼ ***February 9 (Class)***

ASP Work Class

- No new news from CYSF
- Made final edits on procedure section of paper
    - Sent to Dr. Garcia and Fahad for feedback
        - Fahad has left us comment on the document and we will make the necessary edits
    - Document link: https://docs.google.com/document/d/1BlfzZ2WkrblYYKXVE0Q8czXcN0wT-XlJukCsyWYtlDg/edit?usp=sharing
- Edited our graph to look accurate and more clear to viewers
    - Approved by Fahad and Dr. Garcia
- Continued working on poster layout
    - Began working on the background and hypothesis sections of the poster
    - Link: https://docs.google.com/drawings/d/1dV3_FCtk4dLoF4R2aczKBSFOWi1BXRvdmyToV21Bahw/edit?usp=sharing

▼ ***February 13 (Class + Check-in)***

ASP Work Class

- Final edits to the procedure were made before the submission
- Revised all of Fahad’s comments to the procedure
    - Link: https://docs.google.com/document/d/1BlfzZ2WkrblYYKXVE0Q8czXcN0wT-XlJukCsyWYtlDg/edit
- Worked on poster
    - Completed results, acknowledgments, and references
    - Only right side of poster is left: conclusion, significance, future directions, etc.

Check-in

- How do you make sure that your images are representative, explain the qualitative data and recognize that it is not as accurate quantitative
- critize your work, just as examples, will quantify, will redo images of experiment to obtain real numbers
- similar things to poster can be used for the presentation
- Results section of poster: separated into viability and icc

- Understand your statistical analysis well (2 way anova, post-hoc test?)

▼ ***February 20 (Class)***

ASP Work Class

- Sent poster to dr g and mentors
- Final edits on the poster and started to prepare for the oral presentation.
- Researched poster printing companies (comparing prices, printing time)
  - Decided on little rock
- Reviewed feedback on the poster from Dr. G and mentors and incorporated changes
  - Link: https://docs.google.com/drawings/d/1dV3_FCtk4dLoF4R2aczKBSFOWi1BXRvdmyToV21Bahw/edit?usp=sharing
- Conducted a peer review session of our oral presentation for constructive criticism
- Fine-tuned our oral presentation based on feedback from the practice session
- Created a checklist for science fair day to ensure we have everything we need
- Finalized and submitted the order for printing our research poster
- Researched easels that could be used
  - Link: https://docs.google.com/document/d/12GYj1QCxzz9tRldP3rJ7ix457ZzNsdHeT5B1LzsM8LE/edit?usp=sharing
- Worked on ppt presentation for oral in class presentation
  - Transcribed info from poster
  - Link: https://docs.google.com/presentation/d/1__MBhAUwwDXJrnOG_6KHjRC3i0Qm_apOL6yYcn4VFAM/edit?usp=sharing
  - Completed background, research q's and objectives, hypothesis
  - Reviewed the judging criteria for the science fair to better tailor our presentation
  - Checked the printed poster for any errors or issues

▼ ***February 22 (Class)***

ASP Work Class

- 15 projects getting picked from webber out of 24 projects (gr. 7-12)
- Dr g went over our poster, she left comments and we made the edits based on her suggestions
  - Link: https://docs.google.com/drawings/d/1dV3_FCtk4dLoF4R2aczKBSFOWi1BXRvdmyToV21Bahw/edit?usp=sharing
- 15 minutes presentations (dr g advice)
  - DONT FORGET PUNCHLINES!
  - 3 min: BG, RQ, Goals
  - 8 min: Methods, Results (CORE OF PROJECT); don't be too stiff when explaining, teaching mode, leave room for questions
  - 4 min: Discussion (explain, why?), Conclusion (critical, ALWAYS ANSWER YOUR RESEARCH QUESTION, summary of what you learned, take home message), Future Directions, Significance(?)
  - be confident with your knowledge
  - For slides (15-20(MAX))

- Continued working on the slide presentation for our project
  - Focused on clearly explaining our methodology and results
  - Presented our research on the impact of CBD and Ketamine on neuronal growth and connectivity.
  - Detailed our methodology, including cell culture preparation, drug treatment, and cell viability analysis.
  - Discussed our findings and their potential significance in the field of neurology
  - Reviewed our statistical analysis and discussed how our findings correlate with our initial hypothesis
  - Explored the limitations of our study and areas for future research
  - Discussed the potential practical applications of our findings in clinical settings
  - Planned a revision of our presentation based on the feedback.
  - Continued to refine and practice our presentation to ensure clarity and coherence.
- Link:

https://docs.google.com/presentation/d/1__MBhAUwwDXJrnOG_6KHjRC3i0Qm_apOL6yYcn4VFAM/edit?usp=sharing

▼ ***February 26 (Class + Presentations)***

ASP Oral Presentations/Work Class

- *Brynn's Presentation:*
  - genetic diff between endangered and non-endangered species
  - co2 can increase heat resistance
  - diff genomes
  - environmental stressors can make species endangered. what can we do about this?
  - prevention: synthetic biology
  - control: specis of plant pine
  - manipulated: (endangered vs non endangered)
  - reserach q: are there genetic diff betwwen endanagere dnad non-endangered population
  - determine what genetic diff are
  - increase percipitation and lower soil moisture
  - genomes are different
  - Questions:
    - What does the overlap represent?
    - What can you do with this information do save the whitebark species and should these be saved?
    - How did you account for other external factors than the ones you explored?
      - The ones she chose are the main ones she chose.
    - Are these types of pine in the same area
- *Cooper's Presentation:*
  - synaptic pruning is beneficial during adolescence, but bad later in life
  - repeated mild traumatic brain injuries creates changes in synaptic pruning
  - put confounding

    - antibody picture
    - p>0.05
    - write hypothesis not support
    - include why no diff /sources of error
    - low power
    - high varibality
    - SIgnificance: sez, dep,
    - PUNCHLINE, and be more loose
    - put explaination for cyto
    - Questions:
        - What would cause synaptic pruning to occur later in life?
            - he answered this in the pres
        - why do females experience more negative impacts after RMtbi's rather than males?
            - results are debated
        - Can the lateral impact model produce errors in results because the rat is getting hit at the same place rather than different parts of the head?
            - Maybe, but this model was specifically designed to replicate human injuries, so there should be a certain level of accuracy
- Edited final draft of poster to show to dr. g finally before printing
- Worked on slides
    - Methods were separated into multiple slides (avoid info overload)
    - Everything else is completed
    - Link:

    https://docs.google.com/presentation/d/1__MBhAUwwDXJrnOG_6KHjRC3i0Qm_apOL6yYcn4VFAM/edit?usp=sharing

▼ ***February 28 (Class + Presentations)***

ASP Oral Presentations

- *Vincent's Presentation:*
    - lytic activity from municipal of bacteriophage waste waters pathogenic vs non-pathogenic e. coli
    - Background: multi-drug resistance (MDR)
    - Background: bacteriophage
    - Background: ECOR collection and e. coli
    - Fact: 80% of UTIs from e. coli have resistance
    - Objectives
    - Hypothesis
    - Variables
    - Methodology

    - Results
    - Conclusions
    - Significance
    - Questions:
        - If these bacteriophages work, is it safe for humans to take these viruses to kill the e. coli or how will the virus be administered?
- *Tiffany's Presentation:*
    - compare different kinds of cities and different types of first responders and the type of mental health support that they offer
    - first responders likely have trauma/mental health problems on the job
    - first responders are not reaching out for resources that they need but it is also not very readily available
    - research questions/objectives, methodology
    - results/conclusions/recommendations
    - Questions:
        - Is there a way to acess internal resvources that only the first resonders have and how do u think that would change your data?
- *Mariska's Presentation:*
    - effect of eating a diet with high sugar and higher fats in muscle health/activity
    - Questions:
        - What period of time do u think would show a significant difference like how many weeks would you do in the future for diet exposure?
- *Elliot's Presentation*
    - Effects of a bacteria on autism (sociability)
    - autism may be affected by genes or gut microbiome
    - 23-70% of autistic people experience gas issues (from gut)
    - gut-brain axis (small intestine and brain)
    - 3-chamber test
    - microbiome-based therapies
    - Questions:
        - Why does autism cause bigger/more microglia?
- Science Fair info from Dr. G:
    - Posters printed and ready by the weekend
    - monday
    - arrive at school go straight to table in PAC
    - On the stage
    - put poster up
    - 9:00 AM MOST OF the judging starts (Be ready for 8:45)
        - 8:45 latest be in the PAC
        - some judges might come early

        - arrive to put up poster and prepare at reg school time
    - 5 judges/presentation 20-30 minutes each
    - dont have to follow the same script each time
    - score is an average from judges, adjust between them to fit the judge
    - some judges might ask a lot in a certain area, but that's okay

March

March 1 (Class + OUR presentation)

ASP Oral Presentations

- *Ashank & Joel:*
    - type 1 diabetes in the gut microbiomes
    - cell in our body release antigens
        - surface: epitope/mimotope
    - manipulated: orgion of sample (type 1 or not)
    - responding: diff in metabolic pathways between samples
    - RQ1: metabolic pathways and gene differences
    - RQ2: identity potential mimotopes
    - Hypothesis was supported by data and previous literature
    - Conclusion: there is a correlation between certain metabolic pathways and type 1 diabetes
    - Questions:
        - What does the engraftment mean? Why do you want to do this?
            - how well the species stays in microbiomes
- *Amy:*
    - Neurobiological markers associated with psychopathy being predictors of incarcerations
    - extremely skewed violent crime distribution
    - Pre-frontal cortex lack of regulation connects murders and psychopaths
    - Males commit more violent crimes
    - Young people commit more violent crimes
    - Psychopaths are more likely to persist in violent crimes
    - Questions:
        - What biological treatments could treat psychopathy?
            - Not much info that can practically work based off of current info
- *Natalie:*
    - effects of compression garments on heart rate variability
    - no medicatioins to treat pots but compression garments are recommended
    - Conclusion: hypothesis was supported and and compression garment lead to lower heart rate and higher heart rate variability

Class Notes

- Notes on our presentation from dr g:

- slides are good
- looking too much at slides
- explained well
- text is good
- condition of seizure, don't personalize/summarize phrasing
- previous research (lab/literature make clear)
- tighten up for questions outside of presentation
  - why do you apply to cell cultures and not rat?
  - metabolism of cbd process, look into this
  - ed 50
  - look into 2 way anova and post-hoc test
  - seizure induction
  - cbd in bloodstream?

▼ ***March 4 (WA Science Fair)***

WA Science Fair

Judge notes:

- nmda function
- cbd epilepsy
- scale bar and legend on graphs
- behavioural tests for cognitive function instead of just looking at neurons
- what does nmda stand for
- do dead cells have cytosol

▼ ***March 5 (Assembly/Class)***

ASP Class

- We got a gold for our project and got selected for the CYSF!!!
  - Emailed Fahad to update him on this
- Check GC - meeting at lunch tomorrow?
- Steps to CYSF
  - March 15th (Online portal is locked)
  - April 11th (evening, poster setup)
  - April 12th-13th (public)
  - 4 projects from gr 7-8
  - 11 projects from gr 11-12
  - 700-1000 projects at CYSF (one of the biggest in Canada), 12-15 projects get sent to the CWSF
- We will be working on the results section of our final paper next
- Final paper will be due at the end of May (main evaluation of the class)
  - Intro

    - Methods
    - Results (March)
    - Discussion and Conclusions (April, HARDEST SECTION TO WRITE/TAKES A WHILE)
    - Acknowledgements
    - References
- March and April logbooks are collected together
- May is the last logbook
- Final oral presentation, beginning of June (main evaluation of the class)

▼ ***March 7 (Class + Check-in + SF Reflection)***

ASP Work Class

- Results paper advice from dr. garcia:
    - Look at your style of citation (for us APA) to see how figures and tables should be formatted/cited
    - BEFORE your figures are shown in the paper, you need a description of the figure (not an explanation)
- Describing a Figure in our paper:
    - need a title
    - need a figure number
    - Figure 1. Title. (Brief description of the figure)
    - get rid of any titles, textboxes that are not needed/will be explained in the narrative
    - Things to mention in the legend
        - what each bar graph represents, colours, how the error bars came to be, statistics description
    - For the explanation part:
        - average density of this was this, despite this trend, no sig diff to a p value of p,0.05, for this one a similar pattern was seen, with a results of this...
    - Results sometimes mentions the methods, might want to very briefly refresh people's minds in a sentence or two cause some people just skip methodology.
- Ours:
    - Making immunocytochemistry into a figure:
    - take away title
    - talking about 4 images
    - we must label our images (ABCD)
    - can leave control,substanceetamine titles, must refer to those in description
    - Title: Comparison of Immunoflurorescent Staining of Synpatic Connections Between blah blah blah groups.
    - Describing each protein what colour it is. Synaptic connections are shown in yellow....
    - Type of cells that were used.
    - Exposure could be mentioned of the lens
    - magnification of lens
    - in the narrative would be described as:
        - there's no quantification

        - qualitative description
        - describe colours
        - describe what we see
        - more yellow in control than we see in....
        - less yellow in ____ than here
        - describe what we seeing and where it is that located, how much etc
        - no conclusions are made and don't explain why (leave for conclusion and discussion)
    - biggest error in results is showing raw data.
    - never publish raw data
    - ex: photo of microplates
    - wouldn't show stuff before quantification
    - good description of figures is needed, might need to include methods just to give BG info. "When the brain slices were images with.... results were shown in figure 1..."
    - referring to imageJ or graph prism no citations.
    - discussion heavy on citations
    - go through rubrics
    - important: only processed data should be shown.
    - present tense
- Logbooks: April/March logbook is 1, things to add to calendar:
    - Do portal
    - meet w/ mentors
    - divide results section up
    - other paper writing and reading

Dr. Garcia Check-in:

- Questions:
    - For live dead/assay, would we show the images of each group same ones we had in our poster or is that considered raw data since we have a graph for it.
    - Should we show our synapse pictures unlayered, individual colours? for each one?
    - When should we send you our results paper?
    - Science Fair: show judging comments to Dr. Garcia
        - ask abtb splitting up pictures
    - Use softer terms for results
    - tell mentor to fill out mentor evaluation
    - be prepared to ask what a synapse is
    - add ethics to poster?

Judging Comments Reflection (changes for cysf?):

- Judge 1:
    - Good work, split up IF images and add scale bars

    - Background of presentation
    - poster future diections, behavioural tests of mice

- Discussion paper: end of april

▼ ***March 11 (Class)***

ASP Work Class

- Talk to mentors, for improvements/suggestions
- April 30th logbook is due
- I sent a follow-up email for us to meet in person
- SchoolCloud:
    - Organization/Communication (20%)
        - Check-ins
        - Mentor Evaluations
    - Logbook (20%)
        - 15 marks
        - 10% of category
        - 3 left in the year
    - Oral Presentation (30%)
        - One left (50%)
        - Science Fair Judging (20%, gold = 100)
    - Written Assignments (30%)
        - Papers are very critical, marks could go up or down
        - Results (6%)
        - Discussions/Conclusions (20%)
        - Final paper (50%)
- Plan for the coming months and the assignments/goals that we have to reach, now that we have an outlook on our future expectations for ASP
- Results paper will not be marked late until March 22nd, 3:30PM
- Results are simply a description of the results, but not an explanation
    - Marks will be docked for explanations (mentioned in the rubric)
        - Note: when sending mentors our results, send them the rubric as well for context in giving us feedback

▼ ***March 13 (Meeting + Class)***

ASP Meeting w/ Zainab K. (Mentor)

- one person could talk about ketamine, another person could talk about cbd?
- add legend/scale bar
- add arrows to images specify what we are looking at?
    - maybe not
- do dead cells have cytosol?

    - no, but depends on what stage they are in in terms of dying
    - a little bit if not fully dead yet
- cannot make true conclusions on qualitative b/c they are not quantified, maybe say “potential”
- logical extension of the project could be looking at the behavioural impact, we can put this in future directions (think of possible tests)
- step wise approach to any project or process, in vitro testing gives you an indication of whether there is an effect, so you have a rational for doing the in vivo behavioural tests
- like testing for schizophrenia before scanning for mri’s
- number 1 is that the doses that we have used have been clinically adjusted to be clinically relevant, previous literature and are used in vitro
- similar to what is used clinally
- we cannot see how this works through metabolic pathways bc it in in vitro, however these are just first steps
- in vivo studies mimic the exact behavioural aspect
- is there any literature that says ketamine can help epilepsy
- look into how cbd helps epilepsy
- we have 29 days before science fair
- our tasks:
    - revise conclusion, background, scale bars/legends on the poster, add behavioural tests to future directions, send to zainab (& fahad)
    - we should present to people in the lab first, pick a date and time to meet after spring break
    - zainab will send us the scale bars, zz will send her image picture numbers
    - send zainab the graphpad prism data (file)
    - ask zainab about judge who said PBS wash, during that time cells can be lost, damage data
    - ask about difference in intensity of images, how does that affect data

ASP Work Class

- Practiced and filmed video for CYSF portal
- Need to ask dr. garcia how to upload notion logbook into portal