August 25th — meeting @ UofC

First meeting at UofC with Dr Kathy McCoy, & Dr Marcela Davoli-Ferreira.
Basic premise / notes on project.

- autism vs neurotypical (NT) bacteria strain
  - ↓ variations, basically no NT strains.
  - ⇓ mostly the same

[question
↳ how does bacteria strain relate to autism (esp in the brain)]

mice : germ-free { NT, ND } observe differences in social behaviour, health etc (so far results are indicative of hypothesis)

testing : behavioural video clip analysis, dissection of mice brains.

- bacteria releases chemicals? that affects Blood Brain Barrier?
  ↳ affects microglia, neurons, homeostasis, brain development.

readings on: CNS, microglia, gut microbiome, gut-brain axis.

September 8th — ASP class

- set up paperpile extension — citations, literature, reference lists.

Tasks:

↳ email about lab safety course (ask about forms & reading articles)
- proposal guideline.

↳ map out salient topics needed for background reading
↳ make document for all literature, w/ heading system.

↳ read articles previously sent
(host microbiota & microglia, neurodevelopmental disorders)

Article sources: pubmed / frontiersin.org

- annotated abstract of article (microglia and neurodevelopmental disorders — John R. Lukens and Ukpong B Eyo) and section 1.

where are these notes?

September 11th

Marcela emailed for lab safety — Sept 13th at 2:35 @ uofc ✓
- september 2nd — emailed kathy & marcela about spare & ASP hours

September 12th — ASP class

Tasks:
↳ look up 12th grade videos/articles for background
↳ make sure schedule B & acces form are done. ✓
↳ prep topics for meeting tmr (proposal guidelines, lit articles, set up future meetings/next steps (online vs ~~xxx~~ in person), ask about type of project)
↳ logbook & google doc system

due dates?

Dr Garcia meeting:
- ethics form ✓
- schedule! frequency, checkmarks, due dates, next meeting for background research questions. ✓
- proposal: variables, long term & short term goals, experimental.

✓ annotated some of neurodev. disorder microglia article (section 2 & 3)

September 13th — UofC

meeting with Marcela — lab tour, overview of project ✓

current course of action — biosafety → analysis → lab observation

↳ Michael will email about the UCID

analysis → microglial → done on UofC computers
analysis → mice behaviour → done at home, with UofC logbook

bacteria name: clostridium innocuum
- anaerobic
- part of gut flora
- suspected to be a diarrheal pathogen.
- gram positive (essentially a thick cell wall)
- has shown resistance to various antibiotics.

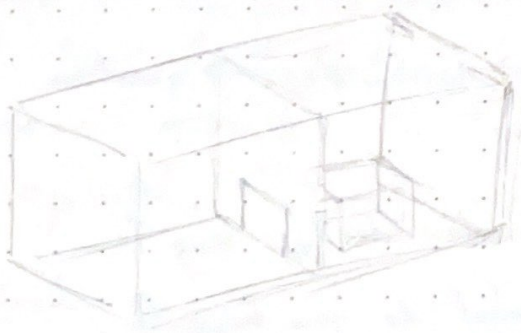
Types of mice used:

Specific Pathogen Free (SPF) — no pathogenic microorganisms, but are healthy. 'normal' bacterial colonies

Germ Free (GF) — usually born from GF parent mice. Kept in a sterile environment, not colonised by any microorganism. No intestinal microbiome, kept in a sterile isolator.

Monocolonised — GF mice that are introduced to one specific microorganism (by researchers) → easy to observe how that isolated microorganism affects the mouse's health

Experiment:
observe differences in social behaviour & brain composition in MC vs SPF/GF mice.



equipment in lab:

- GF hood
- anaerobic chamber (for the bacteria cultured for mice)
  ↳ mice can be dissected in the hood, then quickly transferred to the chamber to prevent oxygen from affecting the gut microbiome. ✓
- autoclave & isolator
- biosafety cabinet
- orbital shaker (incubator)
- international microbiome centre.
  ↳ sterile, shower-ins at facility
  ↳ provided sterile clothing and gloves.

Tasks:
↳ Finish annotating & summarising ✓ microglia & neurodev. disorders.
↳ email Marcela my school schedule ✓
↳ begin reading new articles.

When are these tasks due?

September 14th — ASP class.

class notes.

research proposal
- study
  - data collection
  - no variables
- experimental (likely mine)
  - variables
  - methods
- innovation
  - no research question
  - no variables ✓

proposal outline:

① background research (important aspects)
big question → topics → evidence → narrow down → question

② research question

③ goals → short term (within project window ideally); long term (goals of entire project → what do you hope to achieve with the results?) ✓

(4) methods and variables

☆ significance!

Events:

- Michael Dicay (uofc) emailed myself & the mentors the UCID ALA request form.

Tasks:

↳ email marcela my school schedule for a second meeting ✓

- read article — Fecal microbiota transplantation: review and update by Journal of the Formosan Medical Association
  ↳ Introduction, History, Indications, donor & fecal preparation.

where are the notes on these sections?

September 15th

emailed Marcela my schedule for the week and the ASP application from the grade ten school year. ✓

September 18th. — ASP class

oral presentation tentative dates: October 11th, 13th, 17th.

proposal due: October 6th

- started article: Role of Gut Microbiome in Autism Spectrum Disorder and Its Therapeutic Regulations — frontiers.org (part of introduction)

where are the notes?

- current review, also: Fecal microbiota — delivery methods, challenges, regulations.

Tasks:

by when?

↳ complete fecal matter (JFMA) article. (go over again)

September 19th

Email: UCID Associate - Limited Access approved

Task:

- register for the IT account in the coming days. ✓
- continue Role of Gut in ASD (sections intro & gut-brain axis)

September 20th - ASP class

made a UofC IT and eID account, registered user.

read sample papers provided in class

↳ 7-9 pages ↳ diagrams / visual aids are encouraged.

↳ introductions ~ two / three pages.

- read sections 'effects' of Role of Gut in ASD article. → Notes?
- read research proposal guidelines. in google classroom

Tasks:

→ compile information (relevant) for proposal due dates?

→ email Marcela about the guidelines and general assignment rubrics.

September 22nd — Dr. Garcia meeting

Logbook Rubric:

Organisation

- content < article summaries ✓
  plans for future meetings (points / questions)
- schedule — sept & oct calendars.
  class dates, meeting dates, proposed deadlines.
  communication (email summaries)

- aim for weekly meetings / updates (email / meet etc.)
  ↳ progress, questions, irl meetings, ideas, UCID.

Dr Garcia Meeting:

- email michael diray about biosafety information
- email Marcela — articles & next meeting date. ✓
  ↳ proposed october schedule (e.g. every day 6)

Questions to ask:

↳ mice models (e.g. white mice e.t.c. & how that may affect data)
  ↳ number of mice in each trial (also talk about feeding procedures for context?) ✓
↳ brain function that is being analysed (how the bacteria affects brain tissue & microglia — use of biomarkers / presence or absence of traits?)
↳ specific behaviour of mice (sociability, modes of data collection)
↳ methodology

- read 'maternal microbiota' & 'effect of mode of delivery' sections in 'Role of Gm' article. ✓

September 25th

- gained access to UofC portal., michael emailed the list of compulsory online biosafety courses. ✓
- send pdfs of completion → lab safety & meeting at UofC
- emailed Marcela about the weekly schedule, & questions & proposal. ✓

September 26th – ASP class

- completed 3 biosafety courses ✓
  - ↳ occupational health and safety ✓
  - ↳ hazard assessment training ✓
  - ↳ WHmis 2015. ✓

September 28th – ASP class

- make sure sources are peer-reviewed (reputable org. is not enough)
  - ↳ textbooks
  - ↳ NHI / pubmed (stats/reviews)

avoid institutions → find original sources

- ↳ Google Scholar
- ↳ Health Canada.

★ 'basic' information should still have a citation. ✓

e.g ——— (source1) ———
——— (source 1, 4) –
——— (source 2, 3)

suggested style: APA.
font: 12 pt, Times New Roman.

Calendar

✅ — finished date
🚀 — projected due date
⭐ — deadlines

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
UofC for anymaze				@November 16, 2023		
✅ presentation slides changed				@November 6, 2023		
Untitled						
🚀 finish half current behaviour analysis				@November 16, 2023		
meeting with marcela				@November 14, 2023		
meeting (dr garcia)				@December 13, 2023		
meeting (dr garcia)				@December 1, 2023		
meeting (dr garcia)				@November 21, 2023		
meeting (dr garcia)				@November 8, 2023		
⭐ literature review				@December 15, 2023		
🚀 finish any-maze first batch video data				@November 22, 2023		
meeting (dr garcia)				@October 19, 2023		
meeting (dr garcia)				@October 27, 2023		
✅ revised prop. obj, hyp, method				@October 27, 2023		
🚀 revised prop. obj, hyp, method				@October 27, 2023		
🚀 revised proposal + reference list				@October 30, 2023		
meeting (marcela)				@October 26, 2023		
✅ title and hyp slides				@October 24, 2023		
possibly IMC		@November 10, 2023		@November 6, 2023		
✅ proposal sent (marcela)				@October 22, 2023		

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
🚀 methodology and significance (proposal)				@October 21, 2023		
✅ intro, objective, hypothesis				@October 17, 2023		
🚀 oral presentation title / hypothesis				@October 21, 2023		
meeting (Michael dicay)				@October 17, 2023		
🚀 modified introduction				@October 16, 2023		
✅ annotate c.i article				@October 11, 2023		
meeting (dr garcia)				@October 6, 2023		
✅ intro first para				@October 13, 2023		
🚀 intro first para				@October 13, 2023		
🚀 send proposal draft (marcela)				@October 20, 2023		
meeting (marcela)				@October 12, 2023		
🚀 intro draft				@October 15, 2023		
✅ finished biosafety				@October 6, 2023		
🚀 finish biosafety				@October 6, 2023		
practise presentation (spare)		@November 7, 2023		@November 6, 2023		
🚀 finish presentation slides				@November 4, 2023		
⭐ oral presentation				@November 8, 2023		
⭐ proposal due				@October 31, 2023		
⭐ logbook due				@October 31, 2023		
Untitled						
class: reading abstract and intro	@December 1, 2023			@December 1, 2023		
class: table of data of behaviour analysis	@December 11, 2023			@December 11, 2023		
✅ CYSF portal basic info	@November 21, 2023			@November 21, 2023		

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
🚀 anymaze complete 120 SN (new stranger left and right)	@December 4, 2023			@December 4, 2023		
✅ anymaze 120 SN	@November 16, 2023			@November 16, 2023		
finish making table of results of behaviour	@December 9, 2023			@December 9, 2023		
last day of classes	@December 20, 2023			@December 20, 2023		
class: read section 4.2 of paper	@December 7, 2023			@December 7, 2023		
meeting: IMC and lab	@November 24, 2023			@November 24, 2023		
🚀 anymaze complete 128 SN folder	@December 6, 2023			@December 6, 2023		
✅ CYSF ethics form submitted	@November 27, 2023			@November 27, 2023		
class: check graphs of behaviour analysis	@December 13, 2023			@December 13, 2023		
🚀 check final proposal introduction	@December 14, 2023			@December 14, 2023		
✅ finished slides for presentation	@November 6, 2023			@November 6, 2023		
✅ anymaze 3 videos in 120 SN	@November 29, 2023			@November 29, 2023		
🚀 table of results of 120 and 128 SN and SB	@December 7, 2023			@December 7, 2023		
class: introduction of proposal (add citations)	@December 5, 2023			@December 5, 2023		
class: reading abstract	@November 17, 2023			@November 17, 2023		
🚀 start new video folder	@December 18, 2023			@December 18, 2023		
class: reading section 4.1 of paper	@December 5, 2023			@December 5, 2023		

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
class: reading conclusion	@November 23, 2023			@November 23, 2023		
class: reading abstract	@November 15, 2023			@November 15, 2023		
🚀 anymaze 4 videos in 120 SN (new stranger left)	@December 3, 2023			@December 3, 2023		
✅ anymaze 120 SN and 128 SN	@November 24, 2023			@November 24, 2023		
Untitled						
🚀 graphs for 120 and 128 SN, SB				@December 19, 2023		
✅ 120 and 128 graphs				@December 12, 2023		
UofC meeting				@December 12, 2023		
UofC meeting				@December 14, 2023		
midterms				@January 5, 2024 → January 15, 2024		
🚀 finish graphs for anymaze portion				@January 21, 2024		
🚀 start immunofluorescence				@January 18, 2024		
🚀 finish all female SB videos				@January 19, 2024		
🚀 meeting with Kathy, possibly				@February 21, 2024		
🚀 finish male GF group videos				@January 22, 2024		
✅ finished all female SB videos				@January 20, 2024		
🚀 finish videos and graphs of males				@January 27, 2024		
✅ finished female graphs + male GF graph				@January 23, 2024		
⭐ procedures section				@February 13, 2024		
⭐ science fair				@March 4, 2024		
⭐ presentation				@February 28, 2024		
marks lock				@March 1, 2024		

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
✅ 147 SN folder done				@January 24, 2024		
✅ 148 SN folder				@January 25, 2024		
UofC meeting				@January 11, 2024		
UofC meeting				@January 23, 2024		
UofC meeting				@January 25, 2024		
UofC meeting				@January 26, 2024		
UofC				@February 6, 2024		
🚀 finish IF prep slides				@February 5, 2024		
🚀 intro and background slides				@February 17, 2024		
🚀 compilation of results slides				@February 20, 2024		
🚀 methodology slides				@February 18, 2024		
✅ finished graphs (need to copy ci5a data from other group)				@January 27, 2024		
🚀 127 folder videos				@December 28, 2023		
✅ 127 folder videos				@December 31, 2023		
🚀 127 SN videos				@January 3, 2024		
✅ 127 SN videos				@January 11, 2024		
class — IF videos				@January 30, 2024		
UofC — consecutive for staining		@February 6, 2024		@February 5, 2024		
UofC meeting				@February 8, 2024		
practice presentation?				@February 27, 2024		
🚀 begin IMARIS analyses				@March 11, 2024		
🚀 density and morphology of female ci7 and ci8 groups				@March 21, 2024		

Aa Name	Date	End Date	↗ Related to nov cal priv (1) (Calendar)	Start Date	Tags	↗ nov cal priv
✅ fixed background slides				@February 22, 2024		
✅ significance and variable slides				@February 19, 2024		
✅ marcela — check in				@February 29, 2024		
Untitled				@February 9, 2024		
✅ methodology				@February 14, 2024		
✅ results graphs and slides				@February 24, 2024		
✅ stats of graphs				@February 26, 2024		
🚀 density and morphology — GF female group				@April 1, 2024		

oct 2 c
missed sick class — notes taken from someone else

- calendar / schedule
    - each class (list of tasks) / meetings and reading
    - tasks need due dates
    - summarisation
    - could print out notes

oct 3

- emailed Marcela meeting times on Thursday

Biosafety courses completed: (complete by Friday)

- [x] Harassment and Violence Awareness Training
- [x] Biosafety (Bloodborne Pathogens) Training
- [x] Spill Response Training
- [x] Biosafety (Program) Training
- [x] Biosafety (Annual Refresher) Training

oct 4 c
missed class -appt — notes taken from someone else

- WASF early march
- Dates
    1. Online Portal March 15
    2. CYSF April 18 - 20 (poster)
    3. oral presentation Oct 18 - 31
    4. written proposal Oct 16 - 19
    5. November — work on project

Biosafety course completed:

- [x] Incident Reporting and Investigation Training

oct 6 c / dg

- General comments on logbook: include more detailed tasks and due dates in the calendar
- tasks, date, summaries, emails
- research proposal sections — introduction about 5 - 9 paragraphs
- [x] email free dates

- [x] complete respiratory protection training for biosafety courses today
- [x] email Michael all pdfs of certificates today

Meeting notes:

- Send Dr Garcia dates for next week (will email Marcela)
- max deadline for research proposal is the end of October
- include more things in the logbook (article links, dates, printed certificates etc)

Potential questions ?

- Type, breed of mouse used in experiments (BTBR, shank3b, C57BL/6 etc?)
    - Implications and differences to other mice
- Number of mice used
- Care of the mice — autoclave, feeding, enclosures, isolation practices
- Dependent variables — behaviour of mice and brain analysis?
    - Equipment, technology, and factors of behaviour observations
    - Technology and factors of brain tissue analysis
- Set ups and methodology
    - Diagram of behavioural setup — controlled variables
    - Procedures for brain analysis
        - *When* and *how* are brains collected
            - e.g. XXX days after being mono-colonised
            - Equipment used, measures taken to keep things relatively error-free?
- Possible confounding variables — falsely apparent associations
- How is microglial activation/ maturation measured?
- Methods for c. Innocuum cultures

oct 11 c

- [x] write outline for proposal introduction
- [x] read and annotate Clostridium innocuum: Microbiological and clinical… / Clostridium innocuum: Microbiological and clinical….
- [x] emailed Michael for times to read the lab safety manual (Oct 12, 6pm)
- [x] emailed marcela to confirm time (oct 12 4:45 pm)
- [ ] projected: introduction draft by oct 15
- turnitin: for proposal originality, google classroom: for edits, feedback, and grading (deadline oct 31)
- oral presentation format

    - 10 minutes max for presentation, along with 5 - 10 minutes of questions from the class
    - about 10 - 12 slides
    - explain the terms used! (i.e. strike a balance between knowledgable and accessible)
    - include all sections mentioned in the proposal
    - use graphics!!!
    - e.g. punchline —> background —> goals —> methodology —> question/hypothesis —> significance
    - details of methods are not as important as knowing the general techniques used
    - and also how data graphs can be interpreted
    - flow charts are useful for methodology! esa
- science fair: early march (preferably done with experimental part of project by then)

oct 12 m

meeting with Marcela —

- modified introduction outline:
    1. infant microbiota colonisation (maternal factors and delivery method)
        - Importance (physiological)
    2. gut brain axis and presence of microbiota
        - functions of microglia (neurogenesis, axonogenesis, PCD, immune (inflammatory proteins))
    3. Dysbiosis —> NDD —> narrow down
    4. ASD observed clostridium (links to leaky gut)
        - metabolites and effects on brain
        - aberrant microglia development
    5. previous forms of (biological) treatment — FMT (include pilot), antibiotics
- two tests: behaviour videos and microglia microscopy
    1. behaviour: set parameters, record time that moms spends interacting with cages (anymaze)
    2. microglia —> immunofluorescence —> software program to digitalise —> traces specific parts of the neutron (imaris)
- I could observe the brain slicing / immunofluorescence
- feed mice: Jackson laboratory, mix of wheat / corn / fish meal
- BTBR mouse model
- at least 10 mice for each group, but no set number

- Dysbiosis only applies if one microorganism negatively affects populations of others in the microbiome (i.e. monocolonisation isn't dysbiosis because there isn't a microbiome to affect in the first place)
- c. innocuum is a pathobiont that *may* induce disorders, depending on proliferation / population size
- sociability test: 3-chamber test
- C.i 7 —> neurotypical strain, C.i 8 —> ASD strain (also separated by sex, because female mice have not been studied much before)
- types of mice
    1. BTBR GF — neurotypical sociability (microbiota essential for ASD sociability in BTBR mice)
    2. BTBR Ci 8 — ASD
    3. BTBR SPF — ASD - like
- send proposal draft by the end of next week

oct 13 c

- [x] write first paragraph of introduction based on new outline
- [x] read articles sent by Marcela for the proposal (CIHR proposal, pilot study info, mice conditions in IMC)
- [ ] projected: modified + expanded introduction by oct 16
- WASF March 4 (15 chosen for CYSF)
- March 14 — online portal CYSF closes
- School GC — WASF nuplh2e
- WASF — ethics and basic project
- Poster trifold — no size requirement for pasted papers
    - Can print out a large poster (likely smaller than trifold)
- Possibly make task list in logbook along with the calendar (calendar is proposed deadlines and completion, task list is extension in case dates and completion changes etc. for progress)??

dysbiosis — what it is, how it's caused (maybe give examples of scfa or cytokine. cytokine released by ?, shown to increase microglial activity?) examples of NDD

autism — what it is, dsm five. use article for microglial involvement. prevalence of GI issues, eg leaky gut as a result of dysbiosis. possible sign, and easier for metabolites to go through the intestinal barrier as a result. effects on each other?

previous treatments.

oct 17 m

- Oct 15 and 16 - emailed Michael and confirmed meeting for today at 1pm (safety orientation)

Meeting:

- lab tours
- recycling, fire extinguishers, AED, eye wash
- CHEMATIX — contains all lab chemical inventories and SDS
- went through lab manual again
- ask marcela for SOP (safety operating procedures) for when I go to the lab
- will check UCID card progress
- procedure room — used to impregnate SPF mice, then Fallopian tubes / ovaries are dissected and eggs flushed out
    - ideally embryos are kept till the second stage (after first meiosis?)
    - kept in sterile petri dishes , then transferred down to IMC to be put into GF mice

oct 19 c / dg

- tasks and deadlines, finish proposal sections
- re: oral presentation slides
- Oct 17 and 18 — finished most of literature review, objectives, hypothesis
- [x] finish literature review, objectives, hypothesis, question, variables
- [ ] projected: methodology and significance by oct 21 , title and hyp slide

meeting with Dr garcia:

- oral presentation either November 6 or 8
- ask marcela about the experiment timeline
- for the presentation, make sure to know the terminology / procedures well and also know how different components relate to each other to show results
- eg, IMARIS shows dendrite length etc —> how does that relate to ASD symptoms (a study shows ASD individuals have more dendrites, improper synapse pruning)
- finish literature review, significance and methodology
- use bio render for presentation graphics

oct 22

- sent proposal to marcela and potential meeting times

oct 23 c

- [x] add things to and paste methodology/significance in proposal doc
- [ ] email marcela to meet oct 26 (also need to fill in gaps in the methodology)
- timetables in logbook — calendar, tasks, maybe include ongoing check lists
- November 24 is the cutoff date for term 1 marks — logbook of October and November, mentor evaluation September and October, written proposal, oral presentation, in-class meetings
- presentation: background —> specific research question —> sections in proposal must be labelled —> restate significance
- style: engagement with the audience, flow of speech / presentation, more graphics than text, and any text included must be able to be read clearly
- marcela times: October 26 or 27 to go over proposal
- November 8 oral presentation

oct 25 c

example of oral presentation outline:

1. title
2. general background
3. specific background
    5. research question and hypothesis

methods:

1. flow diagram
2. methods
3. analysis methods

9.-10. significance

- [x] polish significance section
- [x] email marcela to meet oct 26 (also need to fill in gaps in the methodology)
- [x] title and hypothesis slides

oct 26 m

meeting with marcela:

- added suggestions to proposal

- move dysbiosis section up in the literature review
- anymaze protocol
    - add video files and rename
    - group videos that are from the same variable group
    - set parameters of lines, rename parameters, add points and zones
    - experiment tab: add number of mice / number of video files
    - test tab: rescale parameter grid if needed, go to 0:00, and each video will be data-recorded for 10 minutes
        - (if parameter areas don't light up at the beginning of the videos, reset)
    - keys: a is for interaction with the stranger mouse, and z is for interaction with the empty cage
    - data can be seen in the data tab
- after video analysis, marcela will show me how to graph the data
- possibly IMC early November — email times
- Revise proposal before Monday, and could arrange a Zoom meeting to discuss if needed
- next steps: proposal, video analysis, lab observations

oct 27 c / dg

- [x] edit all proposal sections except for literature review
- [x] make background slides for the oral presentation

meeting with dr garcia:

- finish proposal, could send to be checked on Friday / Saturday
- oral presentation — use all the space on the slides! *less text, more graphics*

oct 31 c

- due date for ASP proposal!

- [x] check all references in proposal
- [x] possibly shorten introduction
- [x] check: is research question/goal terminology explained in the intro

Class presentations

Jessica: Design and Construction of CRISPR/Cas9-Mediated Knockout Plasmids for Autism-Risk Genes

- knockout gene —> made non-functional
- determine the genes that cause autism
- knockout plasmid: circular DNA
    - efficient with 2 gRNA systems (improve knockout chance?)
- 400 - 1000 ASD-risk genes
- CRISPR/Cas9 — gRNA attaches to Cas9 protein, Cas9 protein recognises PAM, double-stranded break —> knockout ?
- objective: *designing* knockout plasmid
- independent: vector to insert ratio
- dependent: how efficiently plasmids become competent cells
- design: SnapGene (visualises DNA?) , CRISPR
- backbone (empty) plasmid —> add DNA inserts —> transformed into E.coli DH5a (cells used) —> count colonies
- if complex (not one gene) —> application using plasmids to knock out specific asd risk

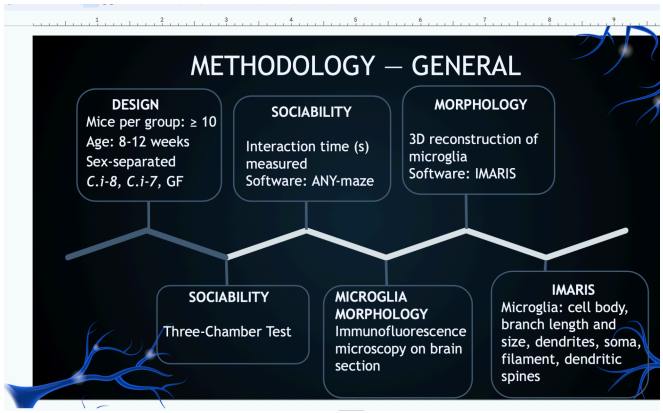
Cooper: Changes in complement protein deposition —> microglia-mediated synaptic pruning after repeated mild TBI in mice

- how concussions affect proteins in brain development (C3 and C1q)
    - changes in complement protein expression
    - effect on microglia density dendrites etc
    - RmTBI mice will have more complement protein (neuroinflammation)
- RmTBI —> neurodegenerative conditions
- synaptic pruning —> microglia assisted by proteins —> removes excess dendrites and synapses —> efficient
- microglia: phagocytose pathogens
- complement system: immune response (opsonisation) —> destroy pathogens
    - C3 — neuroinflammation, pruning in normal brain
        - opsonise synapse, then microglia respond to attack
- studies : RmTBI causes microglia loss —> loss of pruning
- sham injury — control (independent is RmTBI vs sham)
- dependent — density of c3 and c1q
- methods — injury, euthanised, cryosectioning, immunohistochemistry

if complement proteins contribute to opsonisation of synapse —> would rmTBI leads to neuroinflammatory response —> more proteins —> cause excess pruning of synapses??

nov 2 c

- Literature review due December 15
    - the introduction section of the *final* paper (due may)
    - basically proposal introduction + incorporate comments and suggestions
    - or adding more info
- Final paper in may
    - introduction
    - methods
    - results
    - analysis and conclusion
    - early february — procedures and methods
    - end february/beginning march — results + presentation practice
    - april — analysis
    - may — final
- [x] email marcela times for next week and presentation questions
- [x] slides — methodology



- [x] add meeting dates for November and December?
- [x] ask dr garcia about presentation practice (Tuesday spare)

nov 6 c

- November 24 12:00 marks lock in SchoolCloud
- [x] email Marcela — meeting dates, copy of ASP proposal, question
    - [x] 'If the IBA-1 antibody is used as a marker for microglia, will microglial cells be directly analysed? And will the neuronal morphology (spine length/density etc) mentioned in the methodology also be analysed together with microglia in IMARIS?'
- [x] finish methodology and significance slides

nov 8 c

class presentations:

Mariska

- High fat and sucrose diet on muscle health
- HFS —> obesity, hypertension, strokes etc , also sarcopenia (muscle deterioration) — fat infiltration
- Cytokines (MCP-1), macrophages, CD68

Question: how HFS affects vests laterals and soleus muscle in female rats

IHC

How does inflammation relate to fibrosis exactly —> persistent inflammation lead to eventual fibrosis

how would coleus muscle oxidative mechanisms lead to less fibrosis

Natalie

- POTS
- Compression garments — with or without
- Tilt test
- Supine to upright
- [x] presentation

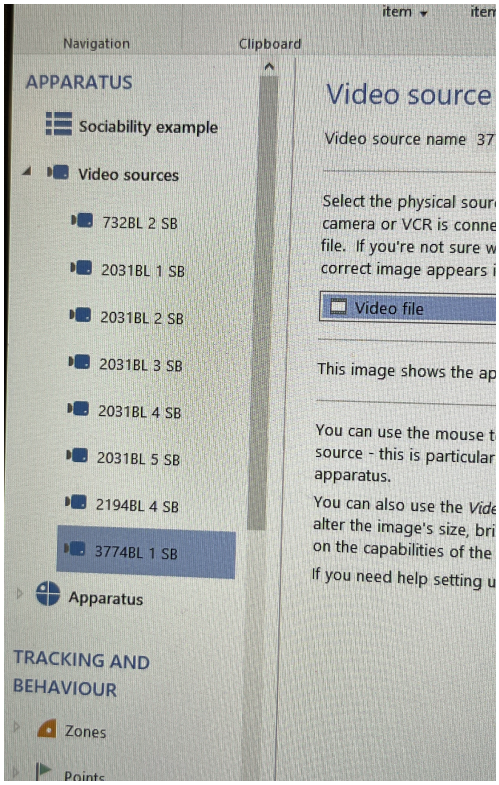
Marcela email — Nov 14 -16 to start behaviour analysis

nov 14 m

meeting with marcela:

- question: whether neurons are analysed using the microglia antibody? — only microglia
- ci8, ci5a
- Thursday for video analysis, leave in 1727
- 4 videos done (732BL 2SB, 2031BL 1 SB, 2031BL 2 SB, 2031BL 3 SB)
    - folder 120 (stranger in right chamber)
    - ci5a and germ free comparison in both folders

- IMC or cryostat next week
- protocol reference for anymaze is in 26/10 notes



nov 15 c

Class presentations

ZZ and Tosin: The Effects of ketamine and marijuana on neuronal viability and function through p2 rat brain cell cultures

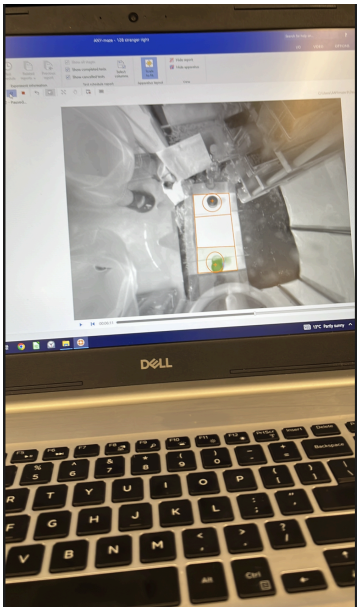
- how ketamine impacts the brain
- neuron cell body, axons, dendrites — synapse — gap between dendrites with electrical signals
- cognitive effects
- ketamine — blocks nmda receptor, found in anaesthetics
- THC compound in CBD oil, responsible for
- ECS endocannabinoid regulates cell communication
- neonate (infant <4 weeks) — compounds like CBD and Ket may affect lifelong development
    - may be affected by mother's intake of compounds

- connection between anaesthetic and long term neurodevelopment
- CBD unclear on how it affects brain (no definitive results)
- question: effects of ketamine and CBD on survival of neutrons (viability)
    - effects on neuronal growth
    - synaptic connections compared to non-exposure to ket and CBD
    - long term: safer medical usage contexts to reduce potential harm
- Hypothesis: exposure decreases cell viability, CBD affects ECS
- incubator conditions kept constant, including co2 and o2 levels
- euthanised rats, hippocampus extracted, hippocampus cultured, cell plating
    - cell viability assay: dyed cells, live and dead cells are different colours
    - IF staining
    - synaptic density, average neurite growth
- 'The **endocannabinoid system** (ECS) is a widespread neuromodulatory system that plays important roles in central nervous system (CNS) development, synaptic plasticity, and the response to endogenous and environmental insults'
- thc negatively affects ecs, how does that actually happen
    - any good effects of cbd on ecs when used in moderation?
    - (in medical condition dosages)

Brynn: Genetic Differences between endangered and non endangered pine species

- connections to climate change
- harsher temperatures, conditions —> loss of habitat —> loss of biodiversity
    - degradation of water quality, altered soil nutrient
- increased biodiversity —> better microbiome exposure in humans (healthier)
    - loss —> also reduced sound barrier
- increase co2 —> increased heat tolerance —> increased photosynthesis
    - but rise in temperature eliminates the benefits of increased photosynthesis
- more temp —> less soil moisture
    - moisture used for dry months is then used earlier —> water deficiency
- prevention
    - synthetic biology — genetically modifications to become resistant to effects of climate change. controversy: accidental creation of invasive species
- question: genetic differences
    - determine specific changes in habitat (predict future habitat, evaluate dispersal patterns, find environmental stressor)
    - gene sequences from pine species
    - long term: change genetic sequence of endangered species
- control: loblolly pine, well studied
    - independent: lodgepole (non endangered), white bark pine (endangered)
    - dependent: type of gene

- methods
    - compile climate variables (map data of temperature, weather etc)
    - species density map
        - find habitat of each species
    - find common stressors
    - repeated, using predicted climate variables (rather than current)
    - create predicted habitats for each species, compare with current habitat models
    - loblolly pine exposed to stressors, use MSA to find similar expressed gene sequences in either species
        - use control to find what the gene does
    - phenological differences
- treegenome.com
- [x] reading: abstract of **Dietary interventions for autism spectrum disorder: New perspectives from the gut-brain axis**



nov 16 m

UofC : analysis on my own

- email marcela if next thursday or friday free for IMC
- video analysis — completed folder 120 SB (sociability)

nov 17 c

Class presentations

Vincent: Effectiveness of lytic activity in phages isolated from municipal wastewater against multi drug resistant e coli and non pathogenic e coli

- 80% UTI caused by MDR strains of E coli
- has antibiotics, but not effective if >20%
- question: can phages from wastewater lyse (break apart) e coli
    - host range of isolated phages (microplane virulence assay
    - tested against 114 + 72 strains of MDR and non-pathogenic E coli
- hypothesis: effective against e coli st131, ineffective against non-p E coli
- Independent: phages / dependent: lysing activity / controlled: incubation time, temperature
- [x] combine introduction with purpose and question

- [x] reading: abstract of **Dietary interventions for autism spectrum disorder: New perspectives from the gut-brain axis**

nov 21 c / dg

Logbook nov 30

CYSF portal

- Log in
- Basic project info
- Ethics (mentors name/email/department of uofc) and code

Meeting:

- Ask how long analysis is going to take
- Need to be done with anymaze and imaris by end of January
- Use February to make poster, prepare for presentations and further understanding
- CYSF if have time
- Research proposal intro no need to shorten
- Make plan for when to finish analysis

Logbook November and December

- PROPOSED calendar for December: predict plans for each class (entries, reading, etc)
- Include intro section December 15 due
    - Add a bit at the end detailing goals and research objective
- CYSF progress
- Reading

Nov 20:

- [x] emailed Marcela — asked about meeting time on Friday
- [x] CYSF portal — basic info section

nov 23 c

class presentations:

Amy:

- indicator of psychopathy
- Uniform Crime Reporting Survey — minor, other, violent
- 18-24 the most violent crimes (crime severity index)
- psychopathy in all crime — 18% to 40%
- common neurological traits between [psychopaths and males, young?]
- psychopathy: nature vs nurture?
    - nature: neurobiological traits, genetics (warrior gene)
    - less activity in gene leads to more psychopathic behaviour
- pet scan: murderer has less frontal lobe activity than normal
- brain scan: psychopath has less frontal lobe activity
- question: psychopathic traits linked to neurobiological abnormalities —> more incarceration for violent crime in males, and young vs old?
- objective: identify biological traits, categorise
- independent: incarceration for violent crime offences, dependent: age, male v female, psychopath vs nonpyschopath
- methods:
    - statistics canada data for 1) young vs old 2) sex —> chi squared test
    - previous literature: psychopathy ratio in English, dutch, Canadian prison —> z test

question : if previous literature of the psychopathy ratio is just English, Dutch, Canadian, could there be a significant bias there — all from the western world ? would the study be best applied to North America / Western Europe only

Joel and ashank

- in silico approach — diabetes in gut microbiome

Tiffany:

- disparities between publicly available vs internally provided into on: psychological intervention available to 1) police officers 2) firefighters 3) EMS responders
- first responders exposed to stress
    - 33% PTSD, 47% depression, 18% anxiety
- epinephrine
    - more heart rate, blood pressure
    - more glucose
    - innate immune system
- norepinephrine
    - alertness
    - constrict blood
- cortisol

- noradrenigc system: under stress, more hypothalamus activity, single to pituitary gland, and adrenal gland, release norepinephrine
- affect hippocampus (dendritic shrinkage and less spine), prefrontal cortex (impulse or recklessness impairment)
- therapies
    - behavioural, EMDR, SSRI
- [x] email Marcela: confirmed Friday meeting
- [x] reading: conclusion of **Dietary interventions for autism spectrum disorder: New perspectives from the gut-brain axis**

nov 24 m

UofC meeting with Marcela

Video analysis:

- [x] finished video analysis of folder 120 and 128 SB
- new folders: 120 and 128 SN (new stranger + old stranger)
- refer back to same labelled video on the empty cage folder, mark down which side contains the new mouse
- change protocol label names (zones, points) and the key name
    - 'empty' chamber —> '*new* stranger left/right' chamber
    - key labels:
        - 'a' — changed from stranger to new stranger
        - 'z' — changed from empty to old stranger
- bring laptop back before Wednesday
- next Thursday and Friday — maybe IMC?

IMC:

- mask, tyvec suit, hair net, socks
- gloves — worn over tyvec suit and taped
- (piercings and glasses have to be taken off / autoclaved before use in IMC)
- change shoes before entering/exiting a room
- 'types' of mouse (colonised with different strains) must be kept in different isolators (not just cages) to reduce risk of cross-contamination

nov 27 c

- Logbook for November and December: add reading schedules (more theoretical background knowledge)
    - And also correct introduction section
- Main focus: data collection and deeper understanding of methodology
    - 2-3 days a week
- November 30 logbook
- 3 weeks till December 15 — how many tasks dedicated towards intro?
    - Internal deadlines, specific
- Schedule for December and January — arrange (esp winter break)
    - Send marcela calendar for December
- Have till mid feb ish (for graphs finish, not data)
    - How does it fit/not fit with hypothesis??
    - If doesn't fit, how to check again?
    - How does literature support that ?
        - Etc
    - Critical analysis of data
- Science fair 4th of march
- Oral presentations in February
- email marcela + ethics code
- For cysf portal — emphasise why sacrifice of mice are necessary

Marcela's email: continue on videos, graphs of data results next week

- [x] emailed Marcela: meeting dates, ask about bringing the laptop back on Tuesday
    - replied: will send ethics code tomorrow, no meeting this week, start results graphs next week ?
- [x] CYSF ethics form sent
- [x] video analysis 1 video: 120 SN (*new* stranger right) (2194BL 3)

nov 29 c

- Oral presentation: February 28
    - Entire project (including conclusion and discussion etc)
        - Does the results match hypothesis? If not, why? How can we fix this? Future direction?
    - 15 minute time limit
    - MUST add at least a minute of elevator pitch at the beginning — explaining what the project is about, explaining the title

    - Rigid deadline, no flexibility
    - Only mark for webber science fair is judging (gold, silver, bronze) — CYSF qualification does not impact it
- [x] video analysis 3 videos: 120 SN (*new* stranger right) (732BL 1, 2194BL 1, 2194BL 2)

dec 1 c / dg

Class notes:

- Each week add tasks (about 6 weeks)
- Logbook, reading, lit intro, data
- Allocate time for each task
    - Reading intro section of paper (30 min)
    - Video analysis — 2 videos (60 min)
    - Read methods of
        - Paper 1 (15 min)
        - Paper 2 (15 min)
        - Paper 3 (15 min)

Meeting:

- What do the graphs look like for anymaze? scatter bar graphs for each group + maybe screenshots of apparatus setup.
- And imaris? scatter bar graphs of numbers + imaris visualisations of microglial cells (to show branch length etc)
    - What would the graph need to look like to be consistent? Significant?
- Finish video stuff by the end of December ideally
- Estimated 40 hours of video data
- What does the imaris graph look like
- Understand how to interpret graphs
- [x] 3 videos of 120 SN (732BL 2 SN, 2031BL 1 SN, 2031BL 2 SN)

dec 5 c

Class notes:

- Next biweekly check, include plans until march
    - Estimate time for each visit, time frame, emails, which week done
    - Poster prep plan as well

- Talk to marcela about long range plan

For the Notion calendar:

Duplicate private monthly calendar, go to table view —> copy all —> ‘move to’ public calendar

In Calendar, copy all the new dates and paste them to start dates (calendar only displays the start dates)

- [x] read article intro + experimental: Altered Gut Microbiota as Potential Biomarkers for Autism Spectrum Disorder in Early Childhood
- [x] videos: 2031 3 SN, 2031 5 SN, 414 3 SN

dec 7 c

Class notes:

Plan for science fair for next biweekly check

December, January, feb —> science fair

- Big steps deadline
    - Smaller sub-steps deadlines
    - Include presentations in class, poster-making (design and creation and printing)
- Plan each week between now and end of February
- Not meeting dates
- [x] videos: 2031 5 SN, 2194 4 SN, 3744 1 SN

dec 11 c

Marcela email — compile data of the video results (copy from anymaze laptop to hard drive in an excel file); mike will find the ethics code for CYSF

- [x] videos: 534 2 SN, 534 3 SN, 3252 2 SN, 3477 2 SN, 414 2 SN (half)

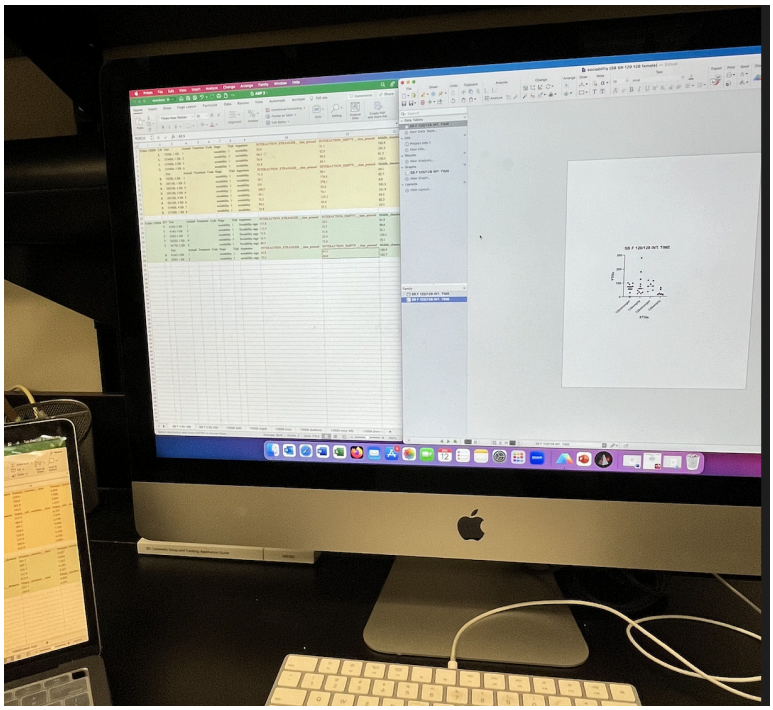
dec 12 m

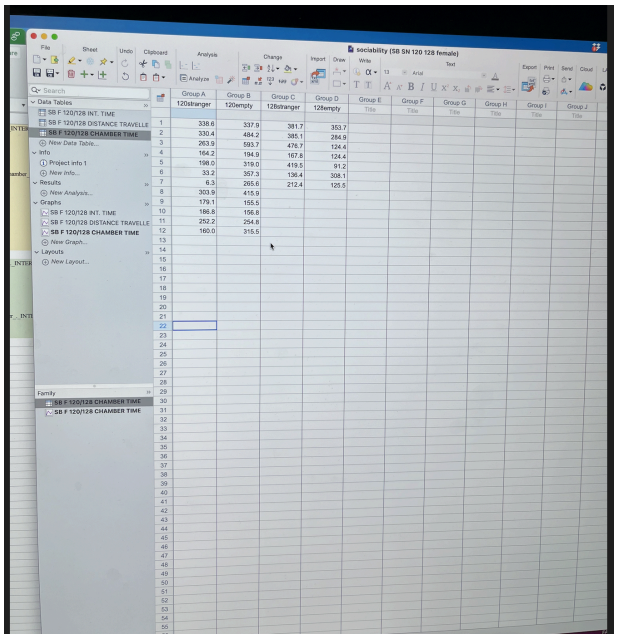
UofC for anymaze analysis

- [x] email marcela about: ethics code, the videos that weren't in the file
- [x] send a copy of the excel sheet so far

confirmed thursday 1:30 to continue graphs

did prism graphs of 120 and 128 SN and SB





dec 13 c / dg

- Estimate time for each visit, time frame, emails , which week done
- Poster prep plan as well
- Big steps deadline
    - Smaller sub-steps deadlines
    - Include presentations in class, poster-making (design and creation and printing)

Meeting with dr garica

- Showed the calendar plan for dec to march
- Should probably practise poster presentation a week before science fair (get done by then)
- Ethics 2B form
- [x] email marcela: missing videos in the 120SN group, excel table of results of 120 and 128 SB, sent significant risk form
- [x] read results + microbiota changes: Altered Gut Microbiota as Potential Biomarkers for Autism Spectrum Disorder in Early Childhood

dec 14 m

- uofc — revised graphs of 120 and 128 SB, labelled axes, changed group names to Germ-free and Ci-5a.
- added new folder of analysis for male group — 127

dec 15 c

Work period:

- [x] email marcela about citations for the introduction paper
- [x] videos: 2343g 1, 40 1 CONT

dec 19 c

Work period

- [x] videos: 2343g 2
- [x] Midterm review

- [x] read abnormal microbial functional analysis: Altered Gut Microbiota as Potential Biomarkers for Autism Spectrum Disorder in Early Childhood

jan 8 c
work period, midterm review

jan 10 c

- work period — watched anymaze videos
- 414G 1 SB (video that wasn't in 128 folder before)
- Retested 414G 2 SN HALF
- 414G 1 SN
- To do : retest 534G 1 SN
- Questions for marcela: 2434g 2 is only 9min 20s, (corrupted files of others), half of 414G
- [x] recover corrupted files (40 1, 40 2, 488 1, 488 2)

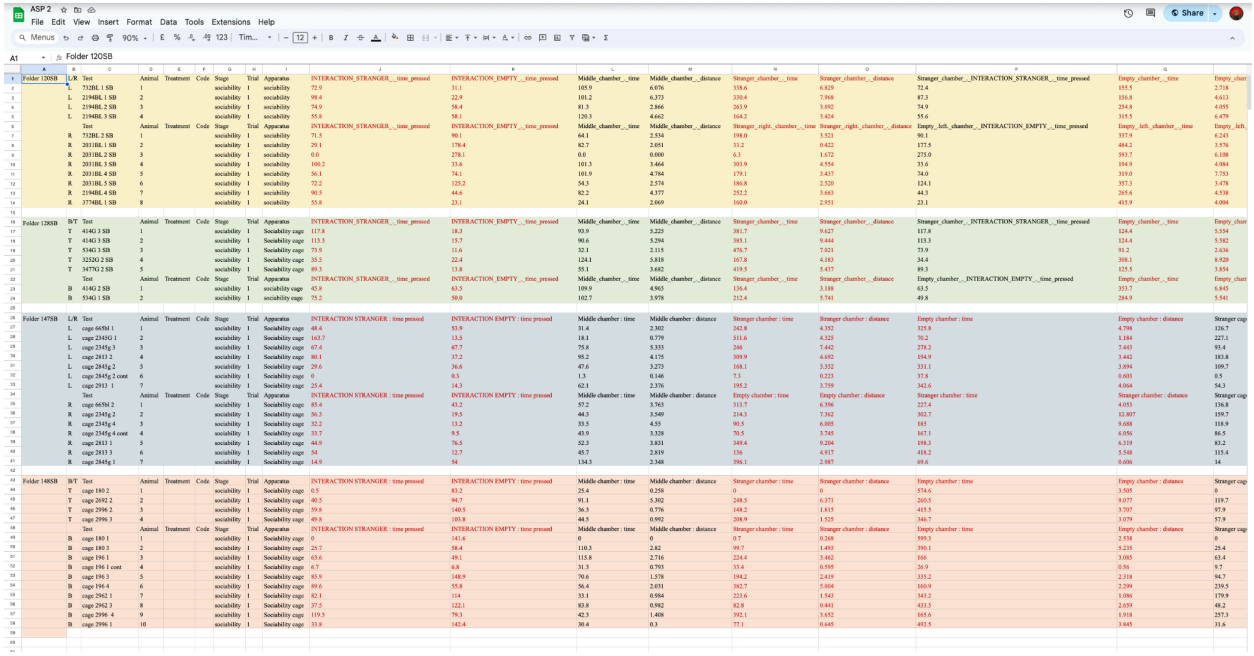
jan 11 m

- Meeting with Marcela:
- Plan for the week after next's Monday or Tuesday for the cryostat, then consecutive 2 days for immunofluorescence prep (3 total)
    - Finish all females (added folders 147 and 148)
    - Fix videos
    - will send males ci5a groups
    - Send fixed videos?
- finish behaviour in the next two weeks
- Meet Kathy mid February
- prep the solutions for brain and cryostat next week
- (not free next thurs or fri)
- next next monday and tuesday for cryostat (ab 230 to 530 ish)
- protocol, stain etc
- showed me the brains and how i'll be cutting them (horizontal)
- put in sucrose solution to dry
- [x] completed 127 SN video folder (male GF group, 7 mice)

jan 23 m

- UofC lab — ANYMAZE and plotting graphs

    - transferred SN videos for 147 and 148 (female all done)
- over midterms, finished 147SB and 148 SB folder (the rest of the female groups)
- Made graphs of all female SB
- groups included: germ free, ci-7, ci-5a, ci-8
- Made graphs of male SB (germ free,) (ci-5a raw data will be taken from Marcela)



jan 24 c

class notes:

- Jan 31st — dec/jan logbooks
- Feb 12 — procedures section deadline
    - What was done, how it was done (not why)
    - Subsections — ethics procedures, no figure results
    - Citations — source (data sets)
        - Name manufacturer (kits, equipment)
- March 1 — marks lock at 9am

Submit intro to turnitin as well

For narrative part of poster — refer to figures as numbers — full narrative description above graph (e.g. figure II describes XXX)

- [x] class and home: complete 147 SN folder (12 mice)

jan 25 m

UofC

added videos:

SB and SN for: male **ci8** and **ci7**

(Male ci5a use raw data from previous testing)

Formaldehyde then 30% sucrose, then freeze brains after

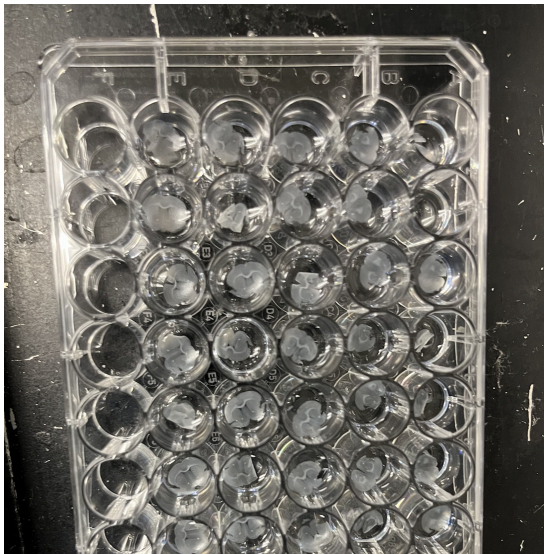
- pipette PBS, 4 plates
- brain samples: froze and cut
    - label dishes, use tweezers to move onto tissue paper, OCT base in dish, cerebellum facing ‘sample’ labelled side, cover with more OCT— wait until slightly white, then continue until completely white. make sure to have no bubbles and keep OCT bottle upside down.

cryostat: temperature modulation between -19 and -21, lock wheel and put down knife cover

1. take sample out, know which side is the right side! cut out side-hanging OCT, put sample in the cryostat
2. OCT on disk, cerebellum side of sample stick to OCT. more OCT around sides.
3. fix blade — 100micrometer
4. put sample disk in, long side vertical.
5. fix sample in place
6. turn blade until tissue starts to show
7. change 100 to 40
8. discard first piece
9. put glass slide over, wet brush, stick to edge and put in tray
- tomorrow: transfer male ci8, ci7 SB and SN
    - immunofluorescence start. 2-3 hours incubation

leica cryostat

- [x] complete 148 SN (13 mice)



jan 26 m

1. under microscope, choose sections from each sample (1 or 2 from each row), look for good/wide cortex
2. in a well plate, all sections from one sample go into one well (with pbs). use brush to transfer.
3. well plate on shaker, 150 for five minutes (pbs wash)
4. in two other wells, put more pbs in and transfer the two samples into each. 150 5 minutes (2nd pbs wash)
5. primary antibody, use pipette — 2ml

process microscope early next week, then 2 weeks to imaris

1. Freeze brain
2. OCT, cryostat
3. Put cryostat slice into well
4. Keep cold
5. Take good slices from each sample under microscope
6. Put plate on shaker 5 min at 150 (PBS wash 1)
7. PBS wash 2
8. IBA 1
9. PBS wash 1
10. PBS wash 2
11. IBA 2 (the one with fluorescence)

12. PBS wash 1
13. PBS wash 2
14. Mount slides under microscope
    - Put small dot of gel over each slice, cover slip make sure no bubbles, nail polish around edges, label name
    - label: ‘sample XXX, IBA-1 • AF555, date’ (Alex fluor 555)

- [x] Email times for next week

jan 27

Finished male groups (ci-7, ci-8, GF)

+graphs for male SB

+graphs for females done

jan 30 c

Class notes:

In the paper, include full descriptions and explanations of figures (reference as fig 1 etc) before the actual figure.

Under the figure legend,

- Fig number
- Title
- Description (more simplified version of the one above / add legend etc)

(but not necessarily for the actual poster)

Poster height 152 cm

Fold left and right 80cm

Centre 82 cm

- [x] watch immunofluorescence video processes

https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=video&cd=&cad=rja&uact=8&ved=2ahUKEwjO3One1JmEAxXPKkQIHbcmBU4QtwJ6BAgUEAI&url=https%3A%2F%2Fwww.youtube.com%2Fwatch%3Fv%3DlQ_O-3IId48&usg=AOvVaw0Jera1CLr1KihzXxKk7D-S&opi=89978449

- [x] immunofluorescence procedure article

feb 1 c

- [x] Confirm science fair on google classroom
- [x] Email marcela about imaris

On march 4th, miss the first two classes (email teachers, bio and social)

schedule for science fair

- 815-845 set up posters
- 9 am judging (5 judges)
- teachers, alumni, other mentors etc
- 10-15 minute limit each presentation, then 10-15 minutes questions, ~ 5 minutes between each judge
- 1130: finished

Meeting:

- Email marcela for IMARIS (if possible, do ci-7 and ci-8 groups first for the most important data)

- Can also include process and include pictures of brain slices etc and explain process
- Most important part is understanding project: if I dont finish it would be good to talk about expected results, then if the results aren't as expected then reasons might be XXX and here's how we could explore options to fix that
- explain variation of data for sociability
- For poster methods, add diagrams

feb 5 m

UofC

prepared 30% sucrose solution + 8 PBS plates

feb 6 m

UofC

- cut three brains in cryostat
- prepared 3 more pfa dishes for thursday
- transferred brains to sucrose (replace with 25ml sucrose, label 'suc', put spine thingy in smaller pfa tube)
- marcela sent me an email to send to cysf (send to kathy after reply to ask her to fill the form and cc marcela)
- on thursday — booked cryostat for 1-3:30, will cut the remaining two brains then start IF
- to clean cryostat:
    - blade out
    - wipe glass lens
    - lock wheel
    - take out inserts with the OCT
    - remove remaining OCT from the inserts, put inserts back in slots

feb 7 c

meeting dr Garcia

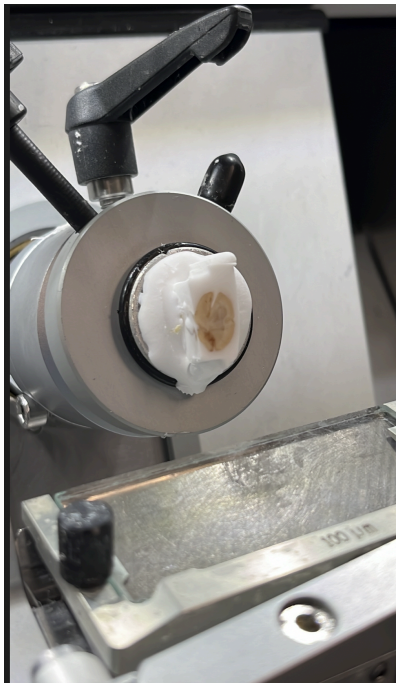
- Collect trifold on Friday
- Organising the poster format
- Reiterated dates for science fair
- [x] methods slides for presentation — anymaze and sociability tests
- [x] email CYSF ethics to check

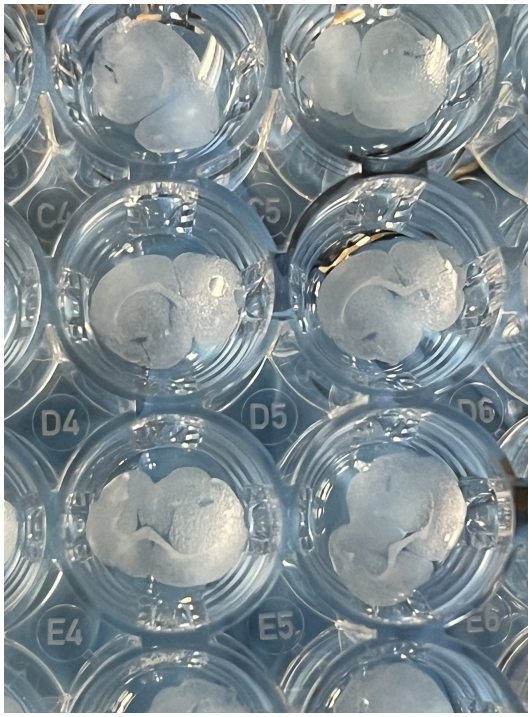
feb 8 m

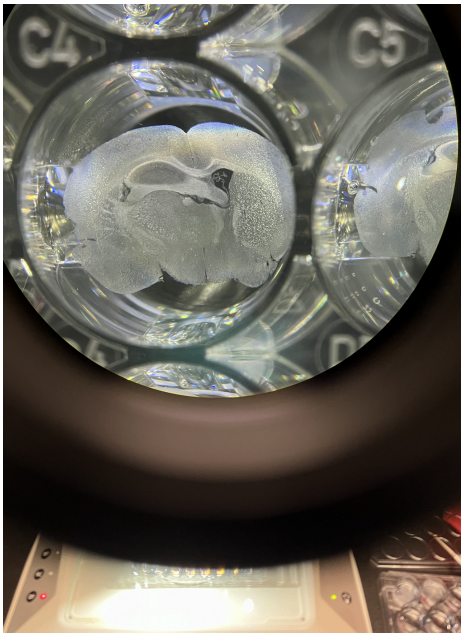
UofC

Cut two brains

Selected 10-12 slices of each brain to put in the well — did this for six brains (64.5, 65.3, 36 (bad slices), 0, 41, and another







feb 9 c

- Informed consent form and significant risk

meeting with dr Garcia

- Adjust background for presentation so that it focuses more on sociability/ implications of bacteria on behaviour
- Rsearch goals: general goal was to examine the role of Ci in ASD — then within that, the main goal was sociability (I.e., take out microglia as one of the main goals)
- Future directions section — make it a more significant part of my poster (30% ish)
    - Could put the microglia methodology in the future directions part in the presentation — or suggest theoretical results that would support hypothesis
    - emphasise ongoing testing

    - Possible results — if it shows [this], then it supports the sociability data, if not, then potentially [that]
        - another future directions section within the future directions section

Remember to allocate more time for this section, after talking about sociability data

- [x] finish sociability part of methodology

feb 13 c

work period

- [x] finished methodology

feb 20 c

Meeting with dr Garcia

- [x] Plan poster outline — background and methods / results and conclusions / microglia as future section on the right
- Set date for presentation practice, Feb 27th
- Introduction/presentation — emphasise importance of sociability ?
    - How it's different from other tests
    - Organismal observations vs molecular and how they could complement each other
    - Know how to explain theory

Email marcela the data and ask for sample microglia data?

- [x] New order of background context — ASD first, then microbiome + gut brain axis, back to ASD observed bacterial differences
- [x] collected trifold
- [x] presentation slides: variables and significance

feb 22 c

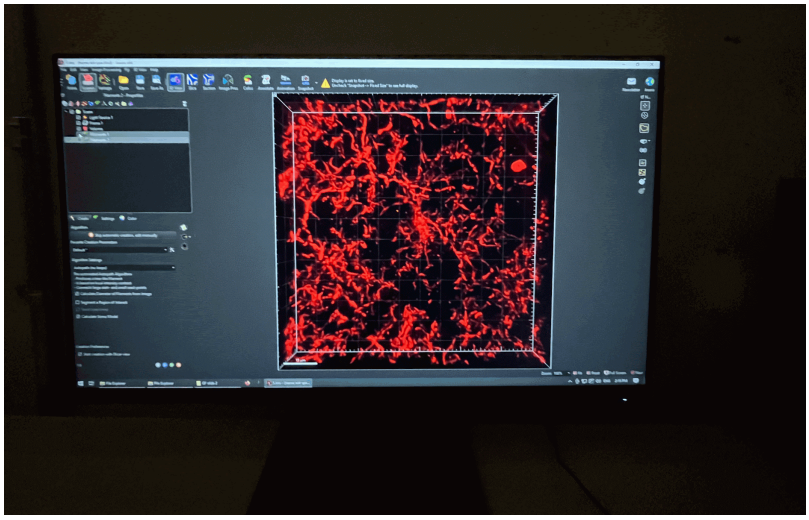
Suggested 15 minutes presentation — 3 min background/research, 8 minutes research and data, 4 minutes significance and future directions Conclusion is important!!! Answer research question, qualify, the take home message

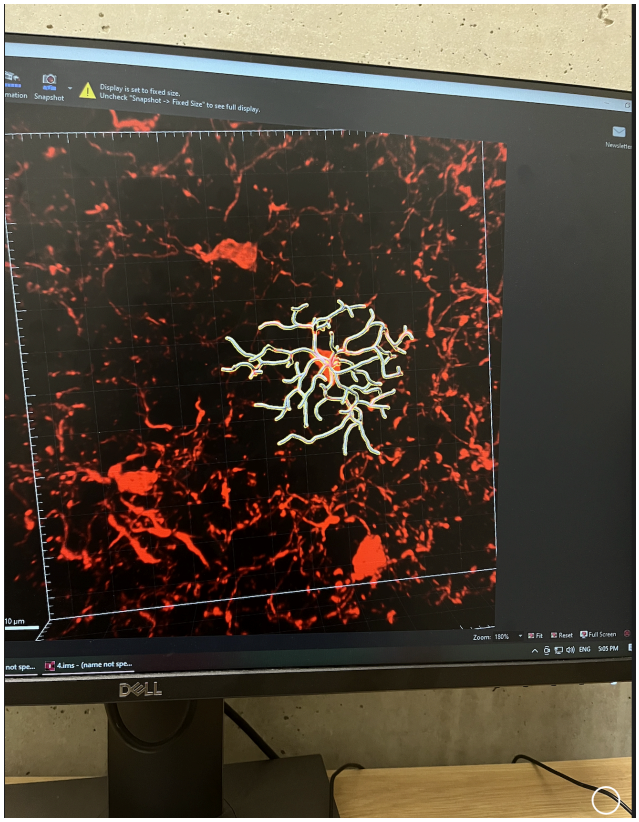
- [x] take out Ci5a from final presentation graphs + reorder and fix axis proportions
- [x] background slides

march 4
School science fair

march 12
IMARIS protocols:
convert tif to imaris
open in the 9.8 version
try to keep seed point value and threshold value the same
delete filaments, branches
for 3, threshold 1.8
in 4, seed 3.4, threshold 1.7

March 14
Images used for IMARIS
GF parameters: seed 3.4, threshold 1.7
GF slide 1: 1_1, 2, 3, 4, 5
GF slide 2: 5, 6, 8, 9
(GF new seed 2.2, threshold 1.17, threshold 5.4)
done march 13 & 14
GF slide 1: 1,2,3,4,5
GF slide 2: 5, 6, 8, 9, 10
(Ci7 seed 2.2, threshold 1.49, threshold 5.4)
done march 14
Ci7 slide 4: 2, 3, 4, 6 (not complete yet)




Snapshot
Display is set to fixed size.
Uncheck "Snapshot -> Fixed Size" to see full display.
10 µm
Zoom: 180%
Fit
Reset
Full Screen
ENG
5:03 PM
4.ims - (name not spe...
DELL