

# Effects of CBD on mRNA markers of hippocampal neurogenesis in the rodent model of Chemo-brain

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## Abstract

Chemotherapy, being the leading way to treat cancer, has proven effective in lengthening the lives of cancer patients. Still, it has the issue of causing several post-treatment cognitive deficits. One of the most widely used chemotherapeutic drugs, Doxorubicin (Dox), can cause several physiological and neurological disorders including cognitive deficits. Cannabidiol (CBD), a non-psychoactive component of *Cannabis sativa*, has been used as a possible neuroprotective agent against neurodegenerative disorders, including Chemo-brain. For the current study, Dox was administered to rats along with CBD given orally. Several rodent model-based behavioral tests were administered to assess anxiety and depression-like behaviors resulting from Dox administration. After completing behavioral tests, the hippocampal tissues were isolated from rats. In this portion of experimentation, we performed RT-qPCR analysis to study the mRNA expression of several markers of hippocampal neurogenesis. The experiment herein will provide insights into how CBD can improve changes in the specific biomarkers of hippocampal neurogenesis following chemotherapy. This also provides evidence supporting our behavioral tests of cognition stating neurogenesis as one of the mechanisms involved in the neuroprotective effects of CBD against Dox-induced chemo-brain. Understanding the mechanism associated with CBD alterations on the behavior deficits related to anxiety and depression in our rat model can provide future avenues for translational studies to evaluate the efficacy of CBD in human chemo-brain studies.

## Methodology

Following the behavioral testing performed previously, the rats were euthanized and decapitated to collect the hippocampal region of the brain. The procedure then followed four steps:

- RNA Isolation:** Total RNA was extracted using TRIzol reagent (Ambion; Life Technologies, Carlsbad, CA) followed by chloroform solubilization and ethanol precipitation. The Nanodrop, a UV spectrophotometer machine (ThermoFisher scientific), was then used to measure the concentration of RNA and it was diluted to 1  $\mu\text{g}/\mu\text{L}$ .
- cDNA Synthesis:** Complementary DNA (cDNA) was generated from 2  $\mu\text{g}$  of total RNA by the High-Capacity RNA-to-cDNA Kit (Applied Biosystem; Foster City, CA). For a 20  $\mu\text{L}$  reaction, 10  $\mu\text{L}$  of 2x RT buffer, 1  $\mu\text{L}$  of 2x RT enzyme mix, about 2  $\mu\text{L}$  of the RNA sample, and 7  $\mu\text{L}$  of free nuclease water were added to a tube. The tubes were then centrifuged and run with the following settings: 37°C for 60 minutes, 95°C for 5 minutes, and 4°C for the remainder. The cDNA prepared was stored at -25 to -15°C.
- Primer validation and RT-qPCR:** Primers were designed using NCBI primer blast tool and purchased from (Invitrogen; Life Technologies, USA). Sequences of primers used are shown in Table 1. Primer validation was done by determining optimal annealing temperatures and PCR efficiencies by using serial dilutions of cDNA. A good primer should have PCR efficiency ranging from 90% -110%, as determined by the RT-qPCR. Melt curve analysis was done to detect the presence of single amplicons without primer dimers. Duplicate samples were prepared for qPCR with 4.5  $\mu\text{L}$  per well of test cDNA, including no RT control to assess the amount of DNA contamination present in an RNA preparation. PCR was performed using Cfx Opus 96, Real-Time PCR System (Bio-Rad; Hercules, CA) and Power Track™ SYBR green master mix (Applied Biosystems). Specific forward and reverse primers, cDNA and SYBR green were added into the wells of PCR plate and run through specified conditions for 40 cycles: 95°C for 2 minutes and 10 seconds, 65-58°C for 30 seconds, and 72°C for 30 seconds. Ct values obtained were taken for the analysis using  $\Delta\Delta\text{Ct}$  method.
- Data analysis:** All data are expressed as mean  $\pm$  SEM. The value of  $p < 0.05$  was considered as the significant difference. With normal distributions, parametric statistical method was used; one way analysis of variance (ANOVA) followed by the post-hoc Tukey's test was used for the multiple comparison when needed. The numerical data was analyzed using GraphPad Prism Version 10 for Windows (Boston, Massachusetts).

## Timeline

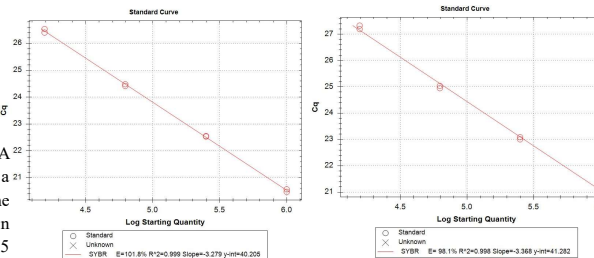
| Week 1          | Week 2 to 5                   | Week 6 to 9      | Week 10                | Week 11 to 13                     |
|-----------------|-------------------------------|------------------|------------------------|-----------------------------------|
| Acclimatization | Dox injections (one per week) | Behavioral tests | Euthanasia of animals  | RNA extraction & cDNA Preparation |
|                 | CBD administration            |                  | Collect tissue samples | RT-qPCR Run                       |

Weeks one through ten were performed previously in the lab and focused on gathering data. This study's focus is on validating gene specific primers and Real-Time qPCR analysis.

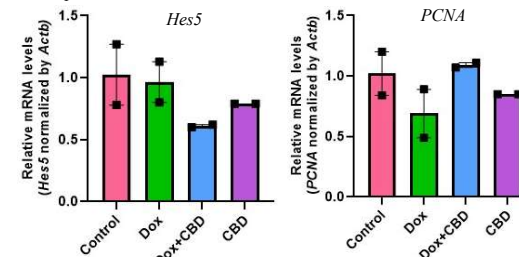
## Results and Analysis

| Gene Name   | Forward Primer        | Reverse Primer        |
|-------------|-----------------------|-----------------------|
| <i>Hes5</i> | AGGAGAAAAATCGACTGCGGA | GCGAAGGCTTTGTCTGTGCTT |
| <i>PCNA</i> | ACTTGAATCCCAGAACAGGA  | TCGCAGAAAACCTCACCCCG  |

**Table 1 - Sequences of primers used in real-time PCR analyses of mRNA expression**



**Figure 1.1 and 1.2 - Standard Curve showing efficiency of the primers (*Hes5* and *PCNA*) respectively. A 4-fold serial dilution of RT was carried out to determine the PCR efficiencies to verify the presence of single amplicons without primer dimers.**



**Figure 2.1 and 2.2 - Quantification of gene expression by qPCR. Relative mRNA expression of *Hes5* and *PCNA* were measured, respectively. The real-time PCR results were standardized against *Actb* and data are presented as Mean  $\pm$  SEM. N=2/group.**

## Discussion

In total, 7 genes were tested in a temperature gradient to test for efficiency of the primers: *DCX*, *Ki67*, *NeuroD1*, *Hes5*, *PCNA*, *Nestin*, and *Actb*:

- *Actb* is a housekeeping gene (always expressed in high concentrations) while *DCX*, *Ki67*, *NeuroD1*, *Hes5*, *PCNA*, and *Nestin* are markers of neurogenesis in the hippocampal region
- Out of the 7 genes tested, only *Hes5* and *PCNA* showed tangible results regarding primer efficiency seen in the graph slopes; between 95-110%.
- The very high and low efficiency could be accounted for by inappropriate concentration and quality of template during the experiments, pipetting errors, incorrect primer design or simply the poor amplification.

Through the analysis of the bar graphs, our results show no significant differences in the mRNA expression of *Hes5* in all the four groups. The mRNA expression of *PCNA* is reduced following Dox, and CBD treated group shows higher mRNA expression of *PCNA*. However, the results are not statistically different to support our hypothesis. If we were to continue the experiment, it would be beneficial to test more animals, try more primers, as well as try genes in a different temperature setting. This repetition of experimentation would solidify data results.

## Conclusion

Although the data does not completely support our hypothesis, future research is important to prove changes in gene expression of markers of neurogenesis following Dox and CBD administration. Designing new sets of gene specific primers, validating them and increasing the biological replicates for each group can be done in the future to further investigate the effects of CBD on Dox-induced alterations in hippocampal neurogenesis.

## Acknowledgements

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# Generative AI and its impact on Public Relations Curriculum

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## Abstract

The purpose of Public Relations is to cultivate the relationship between companies and their target audience. Building these relationships fosters engagement and communication. The PR field is being drastically affected by AI because of its increased use to perform common tasks from creating content to crisis management. With this increased use of AI in the industry, the question is raised should PR students be learning how to use it now. This research focused on how AI is changing the PR field and how PR instructors and students feel about incorporating AI into their course curriculum. We examined instructors’ and students’ perspectives on integrating AI into PR education. Some challenges include ethical concerns and potential job loss, while some benefits are faster work and more personalized communication. PR students can benefit from learning more about AI to enhance their practical skills and critical thinking.

## Introduction

AI is now steadily being incorporated into PR and changing the way professionals collect data, write and deal with the media. While professionals in the industry may have a higher chance of being exposed to it, what about future PR professionals? When it comes to students studying PR, with courses already outlined for the semester, how will PR students get familiar with the new ways AI is being used in the field?

Questions my research answers

- 1) How is AI affecting the ways PR instructors teach their PR courses?
- 2) How do PR students feel about the integration of AI into their PR courses?

## Methodology

Participants: I chose PR students and teachers at Southern Illinois University Carbondale because this sample seemed more convenient. With me already going to school here, I thought I would get faster responses.

Interviews: I interviewed two PR teachers over Microsoft Teams. My first interview was sixteen minutes long and the second interview was eleven minutes long. After the interviews I transcribed them, looked for different themes, and categorized different quotes to the related themes.

Survey: I used Survey Monkey to create my survey. To get the PR students emails for this year I reached out to the Institutional Effectiveness, Planning and Research office. Once I received the emails, I emailed the survey out to the students.

## Findings

Student Survey Results: Due to the lack of responses my survey results were inconclusive.

Teacher Interview: After going over my interviews I found three specific themes. The themes were **human vs. machine, teaching students, and knowing how AI.**

### 1) Human vs. Machine

Teacher A: “Do not copy and paste materials that you know generative AI or chat GPT or whatever it is that they’re using throws out at you and copy and paste it in there. [...] First of all you are not gonna get good content when you do that because it’s not going to be focused because it’s not going to have human thinking behind it.”

Teacher B: “Generally my courses are designed to be a human and to do that, which I don’t think AI is going to be doing anytime soon, relating to one another, offering up opinion and how should things be organized at a wider level.”

### 2) : Teaching Students

Teacher A: “This huge disconnect between what they did in the classroom and then what they find would be the reality out there in the industry. So, we have to be up on [...] teaching students, so the rules and regulations around AI, the legal matters, the ethical matters, maybe we need to develop new courses.”

Teacher B: “We see that AI is creeping into messaging where it’s actually you might have an objective, you might want to accomplish something that you might know to whom you wanna speak, but AI is gonna help you craft the right message to do that. [...] So that the algorithms are getting better and so that’s something that I think we should be teaching in our classes, anticipating allowing it to shape the way we talk to our students”

### 3) Knowing how to us AI

Teacher A: “I think like any other technology that we’ve been exposed to is a double-edged sword and it can be used well and in productive ways to enhance our work and it can be misused as well.”

Teacher B: “ I would say learn more on how it shapes our content and if I were giving advice to PR teachers, I would say always be aware, some of us we want things to be the way they were when we started out. [...] When I started teaching over 20 years ago we were advised to use social media for like let’s say fun things, personality things, promotional things, never anything serious. [...] Of course, that’s night and day [...] and so I think some teachers evolved with social media and in real time. Others like it the way it was and maybe even they liked it before social media was so important, going further back and so this would be similar with AI.”

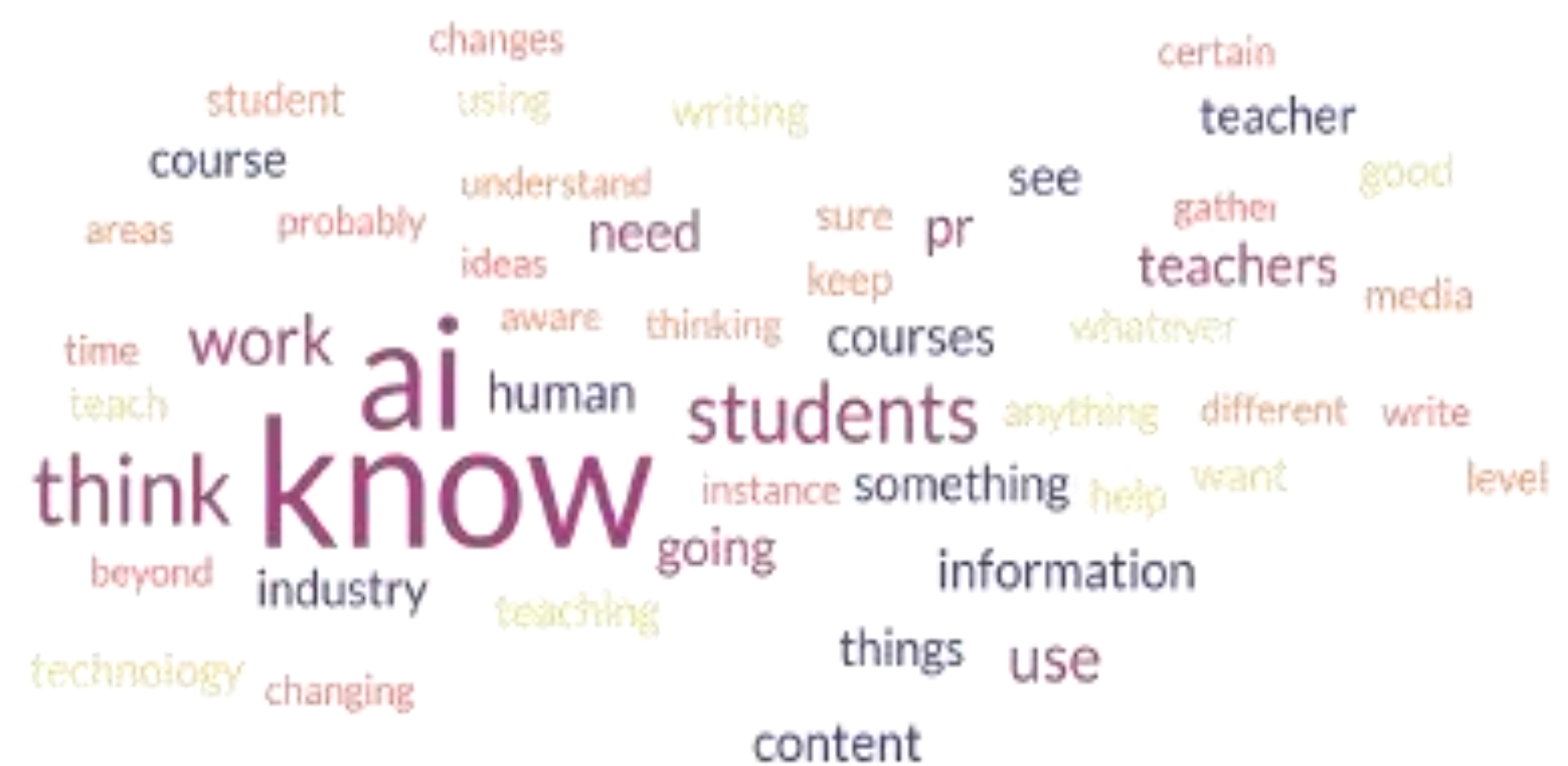
## Conclusion

The goal of my study was to see how much of an impact AI was having on how PR courses are taught. Although the results of my survey were inconclusive, I received valuable insight from the teacher interviews. I learned from my participants that they are aware of the changes AI and would be open to incorporating it into their classes if it means students will be well prepared for the future.

## Acknowledgements

I would like to thank my mentor Dr. Sandy Pensoneau-Conway for helping me throughout this entire research process and always supporting me. I would also like to thank everyone in the McNair Scholar program for always being there to guide me throughout my time in the program.

## Interview Word Cloud



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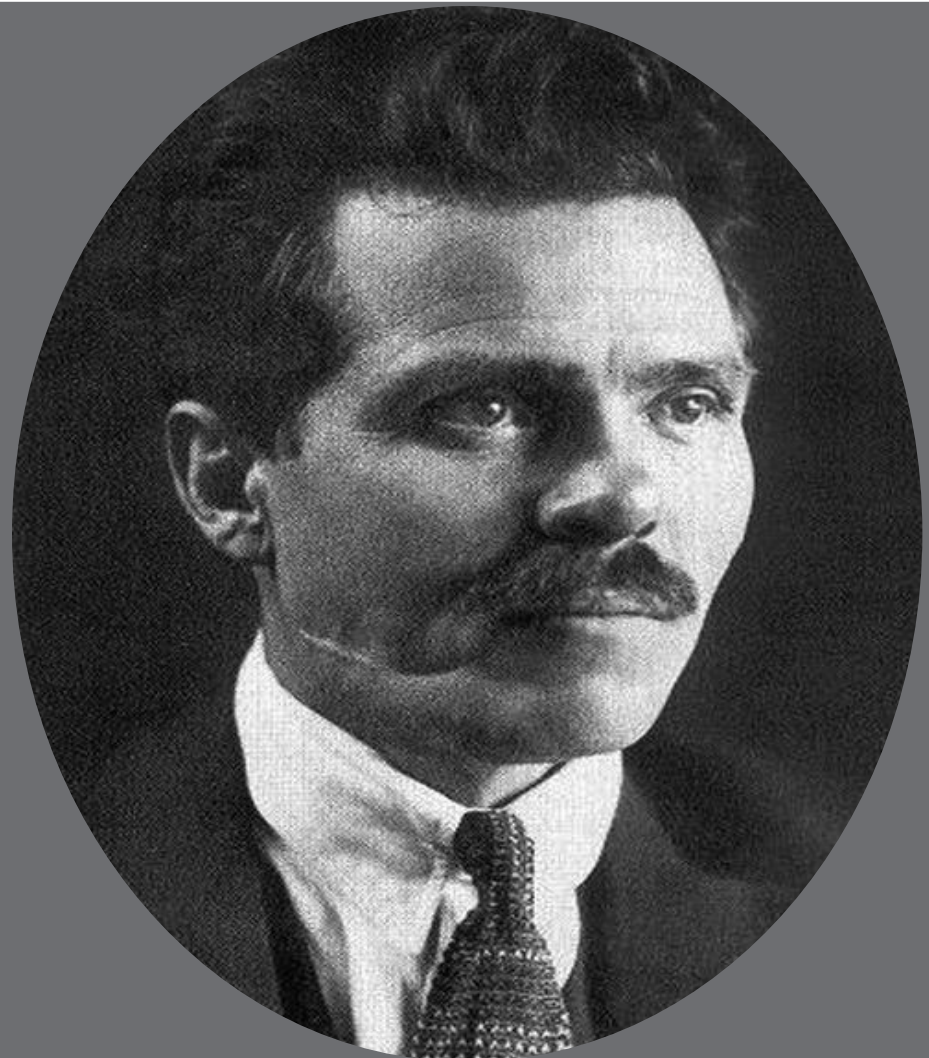


# Nestor Makhno’s Revolution 1917-1921: A Study In Revolutionary Anarchism

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Department of History and Philosophy  
McNair Scholars Program



Petr Arshinov



Nestor Makhno



## Introduction

Between 1917 and 1921 in Southeastern Ukraine, a revolutionary anarchist movement known as the *Makhnovschina*, led by the eponymous Nestor Makhno, battled for its survival. During its war against the anti-communist White Armies and communist Red Army, the *Makhnovschina*’s military protector, the Revolutionary Insurgent Army (Makhnovist) (RIA-M), ranged far and wide over a stretch of land known as the ‘Free Territory.’ In the Free Territory, anarchist ideologues, peasants, workers, and soldiers worked together to build their vision of a better society. Despite these facts, previous research on the *Makhnovschina* has focused on its material culture or its Ukrainian-nationalist characteristics while discounting its anarchist ‘bonafides’.

In my research, I’ve focused on what the *Makhnovschina* represented via its politico-ideological stances and its material policies to answer my research question: What did the *Makhnovschina* represent in the Russian revolutionary period? Answering this question leads us to several implications, most importantly, that there are alternatives to our contemporary normalcy of state-dominated societies, regardless of that society’s economic organization. These alternatives, as they have existed before, should be explored to gleaned advantages that can be used to build better and more whole societies in our present day.

## Key Terms

**Soviets** – *Soviets*, a Russian word that roughly translates to ‘council’ were revolutionary representative bodies composed broadly of soldiers, workers, and peasants. The *soviets* spontaneously appeared after the 1917 February Revolution throughout the nascent Russian Republic, and they acted as both organs of political representation as well as executive authorities.

**Anarchy/Anarchism** – Anarchism is an extant ideological position that argues that all coercive authorities should be extinguished, and local populations should live in democratic communes connected by bonds of comradeship and mutual aid. Though a highly diverse ideological system, anarchism in this period and today differs from communism in that it advocates for the destruction of the state rather than its seizure after the social revolution.

**Makhnovschina** – A revolutionary anarchist movement that emerged in Southeastern Ukraine and that lasted from 1917-1921. It was led by Nestor Makhno and was eventually destroyed by the Communist Red Army in a series of battles between late 1920-1921.

**Nestor Makhno** – Nestor Makhno was a Ukrainian anarchist born in 1888/1889 who in 1917-1919 came to dominate the leadership of the Revolutionary Insurgent Army (Makhnovist) and through the Army the entire movement. He eventually fled the Ukraine in 1921 after the *Makhnovschina*’s defeat. Makhno passed away in 1937 in Paris from Tuberculosis.

## Approach

To answer my research question, I mined several primary sources, including memoirs, eyewitness testimonies, government leaflets, and more for personal and first-hand information on the *Makhnovschina*. Especially important in this regard were the anarchist histories of Petr Arshinov, a longtime comrade of Nestor Makhno and a leader in the *Makhnovschina*. Alongside these primary sources I read several secondary sources from authors like Jonathan D. Smele, Michael Palij, and Michael Malet to better construct the contextual and historical contours of the movement and the period that it existed in.

## Results

- The *Makhnovschina* represented a third direction in contemporary Russian revolutionary politics, weaving between the state-socialism of the Bolshevik Party and the social-liberal-monarchism of the myriad anti-communist White armies. This wholly anarchist direction advocated for two major causes: Free soviets, and free land.
- Rather than seeking state-Party control over the soviets, as did the Bolsheviks, or the abolishment of the soviets, as did the Whites, the *Makhnovists* argued for free soviets. These bodies were thus to form the backbone of a new and free society. This ideological platform descended from anarchist literature written in the 19<sup>th</sup> and early 20<sup>th</sup> century by anarchist luminaries like Mikhail Bakunin or Pyotr Kropotkin who advocated for communities to be united by mutual aid and nothing else. These policies were reflected also in the official propaganda of the *Makhnovschina* (Cultural-Educational Section of the Insurgent Army (Makhnovist)).
- The second plank of the *Makhnovists*’ anarchist policies was its intention to distribute land to ‘nobody.’ That is, the land should be distributed to those who could cultivate it according to their needs and ability to work (2<sup>nd</sup> Makhnovist Congress.) This policy especially descended from Pyotr Kropotkin’s writings on land reform in his seminal *The Conquest of Bread* (Nestor Makhno).
- This differed sharply from both Red and White attempts to intervene in the distribution of land to the peasants and in the oft-brutal grain requisitions sent out from the cities and armies that roamed the Russian countryside (Nestor Makhno).
- Thus, the *Makhnovschina*’s policies concerning the two major questions of political life in the moribund Russian Republic and the nascent Soviet Union, the land and the soviets, were both descended from longtime anarchist ideological frameworks as well as the anarchist leanings of both the Free Territory’s population and its leaders. It was undeniably an anarchist movement.



One of the flags of the *Makhnovschina*. It reads: **“DEATH! To all those who stand in the way of the workers’ liberation.”**



Major territorial extent of the *Makhnovschina*.

## Conclusion

In conclusion, the *Makhnovschina* represented a wholly anarchist direction in the Russian revolutionary period. The core pillars of this direction were the *Makhnovists*’ advocacy for free soviets and free land, a cause that consisted largely of urban and rural soviets that traded and coordinated political and military causes free of coercive authorities. The Free Territory thus represented one of the first real instances of an anarchist polity, and it therefore represented a wholly anarchist direction in the Russian revolutionary period. It was a direction that descended from anarchist ideological frameworks and little, if anything, else. The *Makhnovschina* was not a nationalist expression of Ukrainian independence nor a bandit movement, and it is this originality that allows us to glean from its important achievements new ideas that can be applied to our own societies and our contemporary manners of political construction and expression.

## Acknowledgements

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Ukrainian Farmers



Evaluating Sustainability As A Business Attribute From A Marketing Perspective

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Abstract

- Advances understanding of strategic environmental messaging in social media, focusing on visual and informational cues in environmental advertising.
- Integrates applied psychology insights with social media dynamics to promote environmentally sustainable product preferences, emphasizing the use of logos (life cycle analysis) and information to evoke feelings of pride or accountability.
- Focuses on shaping positive perceptions and behaviors toward sustainability, specifically brand image, purchase intention, and trust
- Aligns corporate interests, consumer behavior, and environmental responsibility, guided by Higgins’ Self-Discrepancy Theory, Social Judgment Theory, and Cognitive Appraisal Theory.
- Surveyed 400 US participants to assess reactions to 2x1 ads with a fictional product life cycle logo, finding significant correlations between pride, brand image, trust, and purchase intention, especially among environmentally conscious individuals.

Introduction/Background

- Environmental Issues:** Human activities harm the environment: pollution, climate change, deforestation, etc. (Grace, 2023); Major contributors: 100 energy companies (71% of industrial emissions) and top 15 U.S. food/beverage firms (630 million metric tons annually) (Axelrod, 2019).
- Incentivizing Environmental Responsibility:** Sustainable consumerism and capitalism require gradual transition; Marketing can unify business and consumer efforts towards sustainability; Psychological factors (accountability, pride) can influence sustainable consumer behavior.
- Self-Accountability:** Motivates ethical consumption choices (Peloza, White, & Jingzhi, 2013); Anticipated guilt can drive pro-environmental behavior (Mallett, 2012).
- Pride:** Influences sustainable behavior by self-worth and achievement (Antonetti & Maklan, 2013); Joy and pride reduce plastic usage (Peenhancingter and Honea, 2012); Pride appeals encourage broad sharing of content (Septianto et al., 2021).
- Life Cycle Analysis (LCA) and Sustainability Logo:** LCA evaluates environmental impacts throughout product life cycle (Moutik et al., 2023); Holistic approach differentiates LCA from other tools (Curran, 2013); Sustainability logos combine images and information to improve trust and label credibility (Wang et al., 2022); Can make environmentalism more accessible, even to less eco-aware consumers; Logos and visual cues influence consumer behavior and perception (Higgins, 1987; Lazarus, 1960).

Hypotheses

- H1:** The inclusion of environmentally impactful framing in social media advertising will evoke high pride and result in an enhanced (a) brand image, (b) purchase intention, and (c) brand trust.
- H2:** The level of environmental involvement of an individual will affect the overall impact of hypothesis 1(a,b,c)
- H3:** The inclusion of environmentally impactful framing in social media advertising will evoke high accountability and result in an enhanced (a) brand image, (b) purchase intention, and (c) brand trust.
- H4:** The level of environmental involvement of an individual will affect the overall impact of hypothesis 3(a,b,c)

Subjects

- 400 participants across the United States on the survey research platform Prolific; (62% female).
- Age: 36 years

Measurements

- 2x1 between-subjects design.
- Two advertisements: one with life cycle analysis logo and one without - randomized per subject via Qualtrics.

- Measurement Tools:**
- Overall, great internal consistency: Environmental Involvement ( $\alpha=.821$ ), Pride ( $\alpha=.939$ ), Accountability ( $\alpha=.908$ ), Brand Image ( $\alpha=.893$ ), Purchase Intention ( $\alpha=.917$ ), and Brand Trust ( $\alpha=.931$ ).
- Measurements were formatted on a 7-point Likert scale (1 = strongly disagree, 7 = strongly agree).

Measurements

- Social desirability bias**
- Utilized a 4-item scale from Crowne and Marlowe (1960).
- Measured on a 7-point Likert scale (1 = strongly disagree to 7 = strongly agree).
- Important to account for individuals responding in socially acceptable ways.
- Utilized Hayes PROCESS Macro (Hayes, 2017):**
- Identifies direct and indirect effects in mediator models.
- Analyzes interaction in moderator models

Image 1: Manipulation 1 (Logo)



Image 2: Manipulation 2 (No Logo)

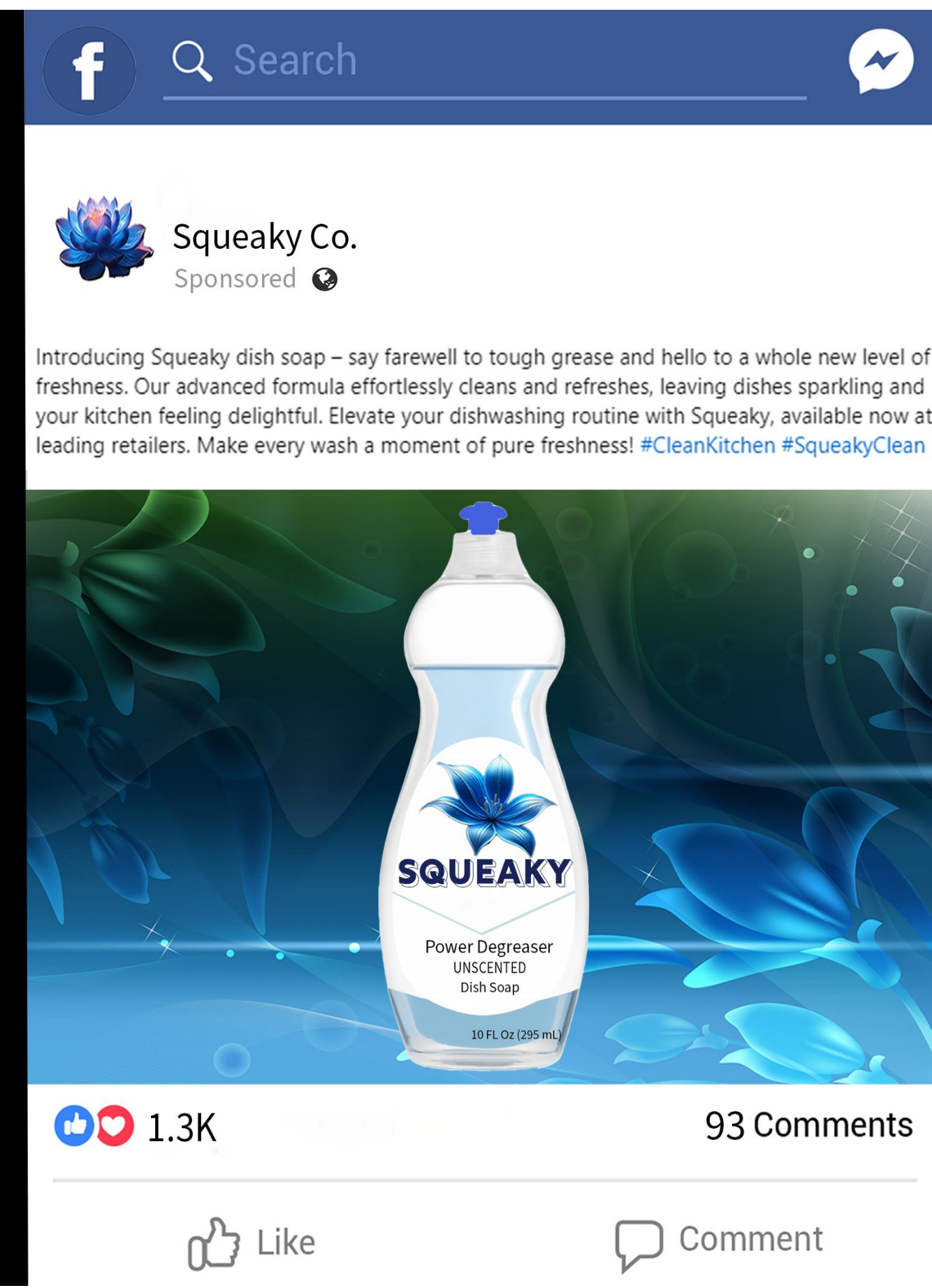


Table 1: Mediation Analysis

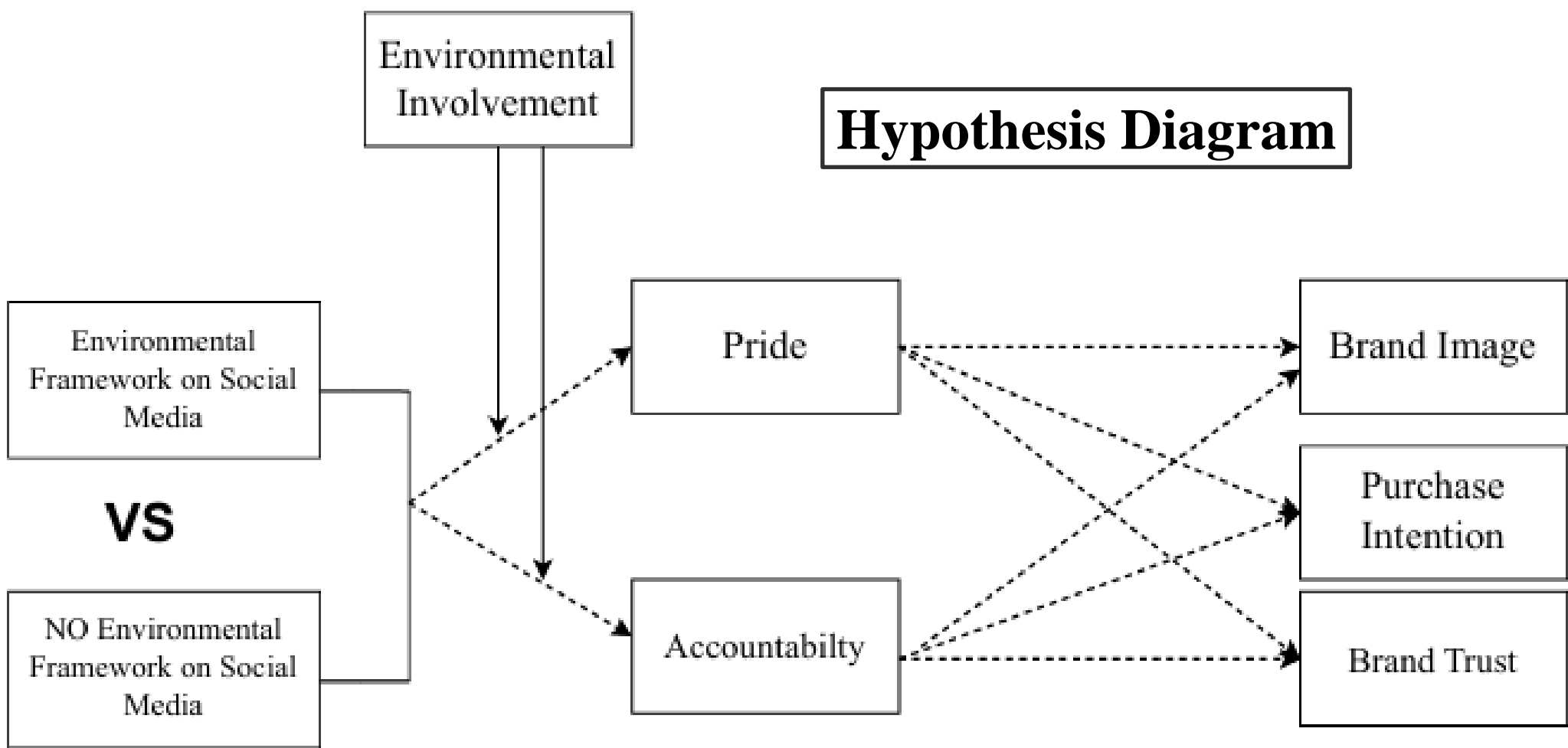
| Indirect Effect    |         |     |               | Direct Effect |          |     |          |
|--------------------|---------|-----|---------------|---------------|----------|-----|----------|
| X -> M -> Y        | Effect  | SE  | 95%           | X -> Y        | Effect   | SE  | p        |
| MANI -> PRD -> IMG | b= -.92 | .10 | [-1.11, -.74] | MANI -> IMG   | b= -.24  | .09 | P < .001 |
| MANI -> PRD -> PI  | b= -.99 | .10 | [-1.20, -.79] | MANI -> PI    | b= -.16  | .11 | P = .16  |
| MANI -> PRD -> TRT | b= -.84 | .08 | [-1.02, -.64] | MANI -> TRT   | b= -.24  | .10 | P < .05  |
| MANI -> ACC -> IMG | b= .06  | .03 | [.01, .13]    | MANI -> IMG   | b= -1.22 | .11 | P < .001 |
| MANI -> ACC -> PI  | b= .04  | .02 | [.00, .10]    | MANI -> PI    | b= -1.19 | .23 | P < .001 |
| MANI -> ACC -> TRT | b= .02  | .02 | [-.00, .07]   | MANI -> TRT   | b= -1.11 | .11 | P < .001 |

Abbreviation: MANI, Image Manipulation; ENV, Environmental Involvement; PRD, Pride; ACC, Accountability; IMG, Brand Image; PI, Purchase Intention; TRT, Trust.

Table 2: Moderated Mediation Analysis

| Moderated Mediation Path  |         |              |
|---------------------------|---------|--------------|
| X -> W -> M -> Y          | Effect  | 95% CI       |
| MANI -> ENV -> PRD -> IMG | b= -.23 | [-.39, -.09] |
| MANI -> ENV -> PRD -> PI  | b= -.25 | [-.43, -.09] |
| MANI -> ENV -> PRD -> TRT | b= -.22 | [-.36, -.08] |
| MANI -> ENV -> ACC -> IMG | b= .04  | [-.01, .09]  |
| MANI -> ENV -> ACC -> PI  | b= .02  | [-.01, .06]  |
| MANI -> ENV -> ACC -> TRT | b= .01  | [-.01, .05]  |

Abbreviation: MANI, Image Manipulation; ENV, Environmental Involvement; PRD, Pride; ACC, Accountability; IMG, Brand Image; PI, Purchase Intention; TRT, Trust.



Results

- This study found a significant indirect effect of pride on brand image, purchase intention, and brand trust (See Table 1).
- This study found a significant indirect effect of accountability on brand image and purchase intention (See Table 1).
- There was a significant moderation effect of environmental involvement on pride overall (See Table 2) (View Chart)

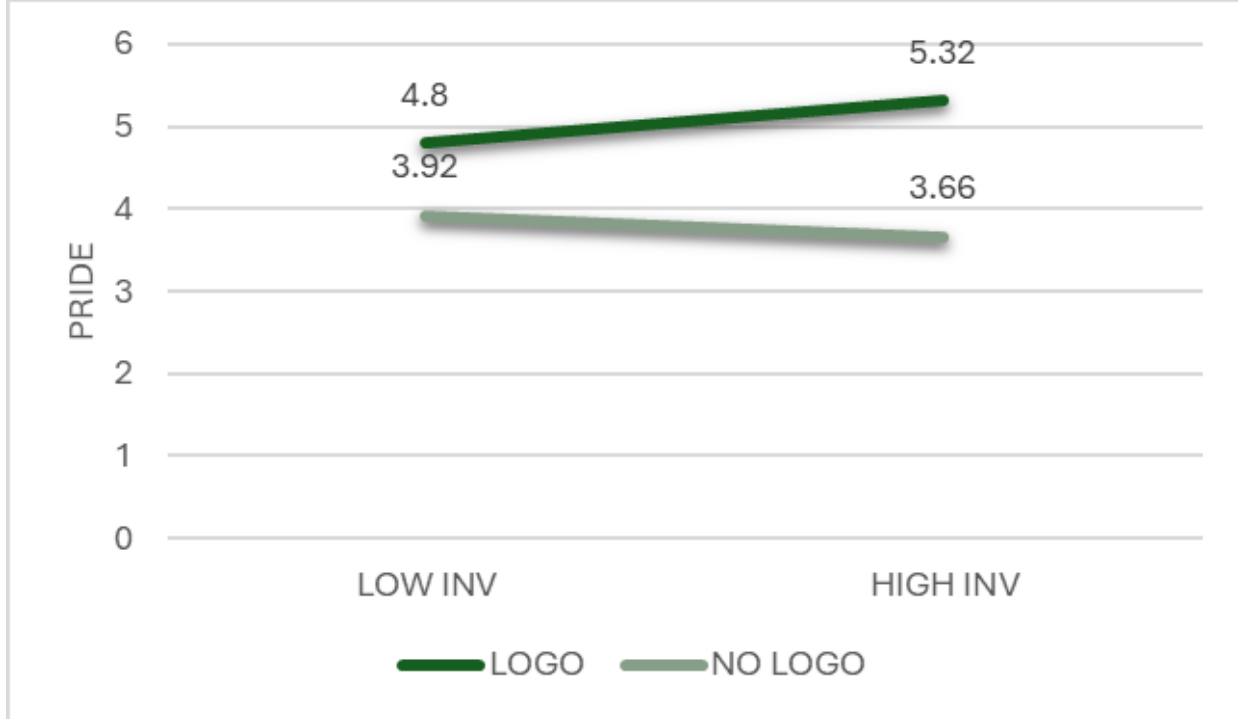


Chart 1

Conclusion

- The mediation analysis shows that Facebook ads evoke pride. This effect is stronger when the ad includes a life cycle analysis logo. All in all, consumers feel greater pride when they see sustainable products, associating them with positive personal actions. This pride significantly enhances brand perception, purchase intent, and trust, especially among consumers with high environmental involvement.
- Despite the literature and theoretical support for accountability (H3 & H4), further research must be done regarding this. There may be an omitted mediator or alternative factors causing this competitive mediation. These variables are significant and, therefore, should continue to be studied.
- Emotions are one of the most transformational ways of influencing society; so, it is imperative to continue studying this sector of marketing for the sake of the planet Earth.

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# Assay development to monitor the affects from antisense suppression of the toxin microcystin produced by the cyanobacterium *Microcystis aeruginosa*

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## Introduction

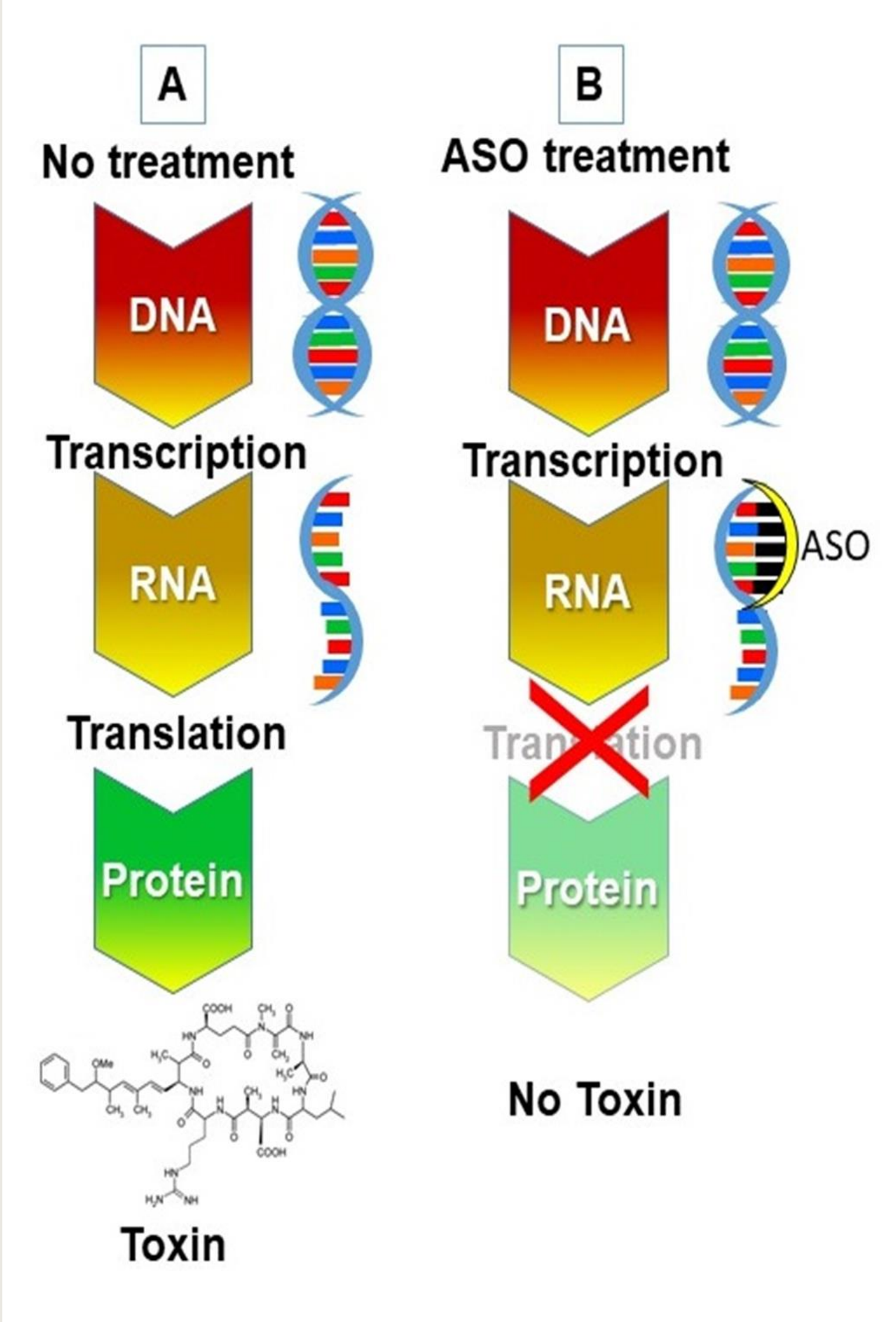
*Microcystis aeruginosa* is one of the primary microorganisms that cause cyanobacterial harmful algal blooms (cHABs) (**figure 1**) and its growth is exacerbated by nutrient runoff from agriculture, stagnant water, and climate change. The ecological impact of cHABs causes millions in US dollars (billions of US dollars globally) in damage per year to recreational, agricultural, and municipal water systems. The current methods for controlling cHABs are underdeveloped and imperfect, resulting in a need to improve current methods or find new approaches.



**Figure 1. cHAB event.** Captured at SIUC Campus Lake in June 2021

Once a body of water is contaminated by a cyanobacterial toxin there is no practical way of removing it. Microcystins typically have a half-life of 4 days to 14 days in surface waters to reach below EPA safe drinking standards <1.6 µg/L for children. Although it is not clear what human health effects occur from chronic exposure to cyanobacterial toxins, studies have shown that multiple sclerosis and kidney/liver damage may result from chronic exposure to cyanobacterial toxins.

There are several well-established methods for detecting microcystin, but despite the variety of approaches, none of them succeed in stopping the production of toxin. The central dogma states that DNA is replicated to create new cells, DNA is transcribed into RNA, and RNA is translated into proteins (**figure 2**).

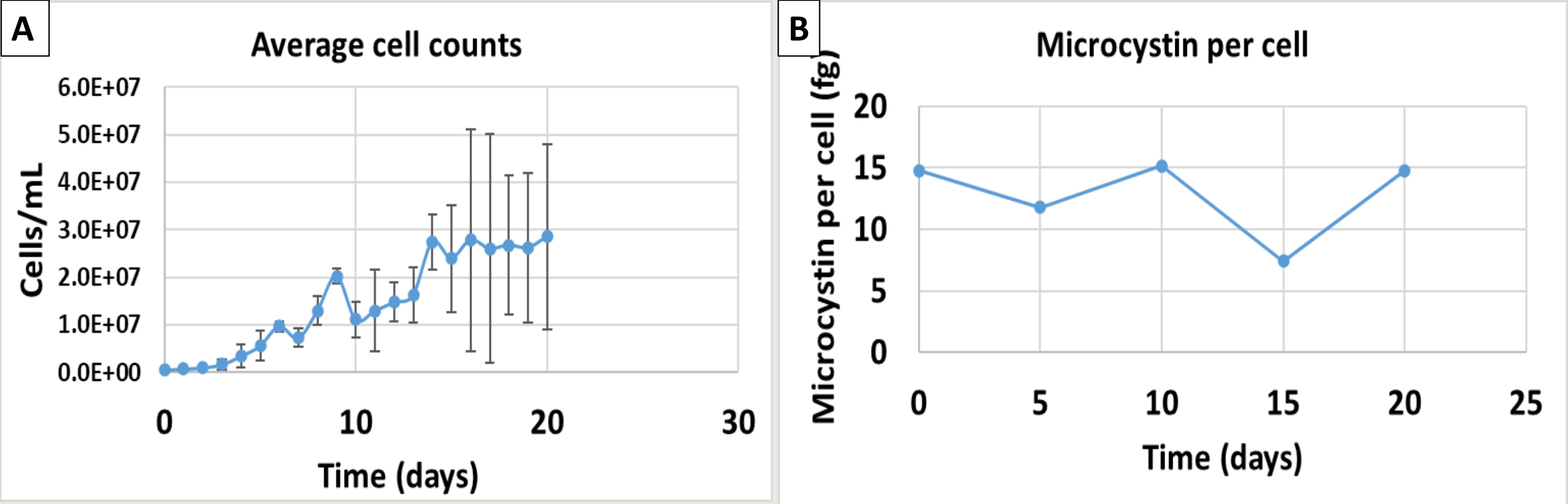


**Figure 2. Concept diagram of how ASO's affect central dogma.** A) typical pathway DNA -> RNA -> Protein, B) introduction of an ASO complimentary to RNA disrupts the process for making protein product.

Antisense oligonucleotides (ASO) can be used to interrupt the process of translation through binding to or degrading the target RNA sequence (**figure 2**). The application of ASO technology in prokaryotic organisms remains underdeveloped, and to the best of our knowledge, ASO technology has never been applied to cyanobacteria. If ASO technology can successfully knock down the production of microcystin in *M. aeruginosa* by interrupting translation, this will be the first study to prove that this technology can be used to achieve these results in prokaryotic organisms.

## Results

*M. aeruginosa* cultures were grown in quadruplicates and monitored daily via hemocytometer to track cell density, generating a growth curve (**figure 4A**). A standard solution of microcystin was analyzed in a range of concentrations to generate a standard curve by which the total concentration of microcystin in all experimental samples was calculated (**figure 4B, 5**). While cell counts using hemocytometer are an industry standard, the variation in counts can be extreme, indicating that this is an imperfect method for tracking cell density.

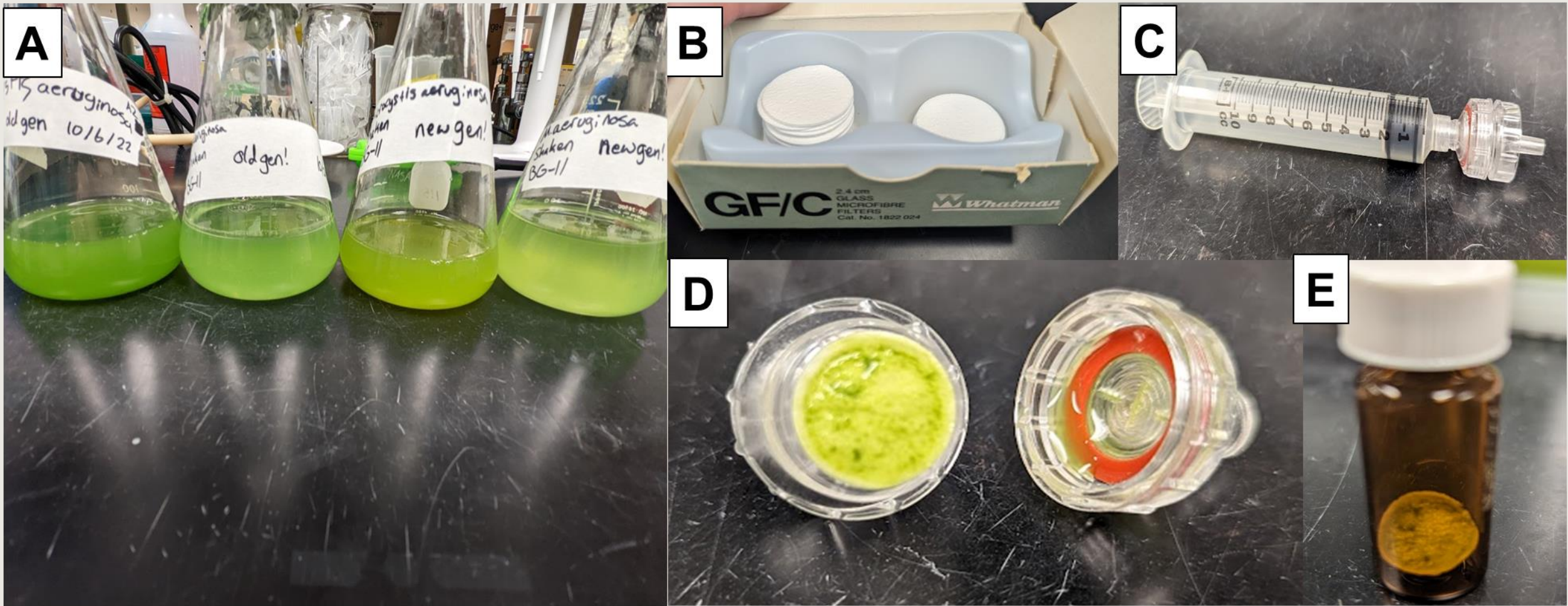


**Figure 4. Growth curve and microcystin content.** A) Growth curve generated from cell counts over 20 days. B) Average microcystin content per cell over 20 days.

## Methodology

### Filtration method and application of QuikLyse procedure

*M. aeruginosa* cultures were grown to a density of 10<sup>7</sup> cells/mL (**figure 3A**). Glass fiber filters (**figure 3A**) housed within filter casing were prepared and attached to a 20 mL syringe (**figure 3B**). Using the prepared syringe, 15 mL of culture (**figure 3E**) was passed through the filter (**figure 3C**). The filter was removed from the casing and placed within a 4 mL glass bottle containing 1 mL of distilled water (**figure 3D**). QuikLyse solution (acquired from Eurofins Abraxis) was added to the bottle in accordance with the procedure provided with the QuikLyse kit. Lysed samples were extracted and prepared in HPLC vials for analysis.



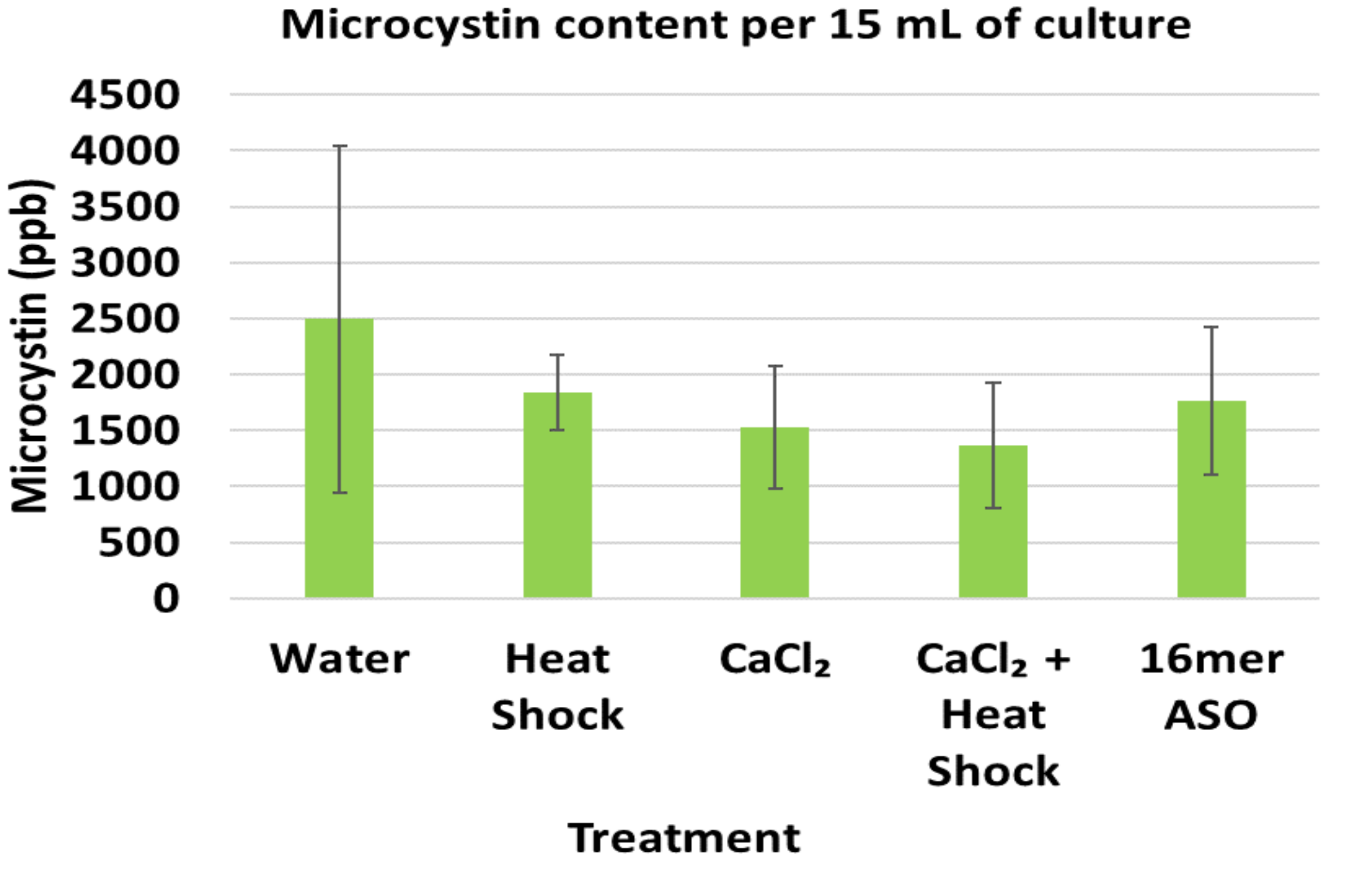
**Figure 3. Filtration method and QuikLyse procedure.** A) *M. aeruginosa* live culture. B) GF/C filters used to capture *M. aeruginosa* cells. C) 10 mL syringe attached to filter casing housing the GF/C filter. D) Filter after culture has been passed through it. E) Filter submerged in QuikLyse solution.

### ASO assay

After *M. aeruginosa* cultures were grown to a density of 10<sup>7</sup> cells/mL, 15 mL of culture was aliquoted in 25 flasks according to **Table 1**. Each sample was centrifuged at 9,500 RPM for 5 minutes. The supernatant was decanted, and pellets were resuspended in 100 µL of molecular grade water or 100 µL of 100 mM of CaCl<sub>2</sub> according to the treatment. Each sample was incubated on ice for 15 minutes. Treatments were then applied to each sample, and they were incubated on ice for an additional 30 minutes. Following this, all samples receiving heat shock were placed in a water bath set to 42°C for 1 minute. Each sample was then resuspended in 14.9 mL of BG-11 and returned to the shaking incubator. After 24 hours, the samples were processed for HPLC analysis.

**Table 1. Experimental outline for antisense assays on *M. aeruginosa***

| Experimental Number | # of flasks | Treatment  |
|---------------------|-------------|--|
| 1                   | 5           | Water  |
| 2                   | 5           | Heat Shock   |
| 3                   | 5           | CaCl <sub>2</sub>                                    |
| 4                   | 5           | CaCl <sub>2</sub> + Heat Shock                       |
| 5                   | 5           | CaCl <sub>2</sub> + Heat Shock + ASO <sub>mcyE</sub> |



**Figure 5. Results from ASO assay.** Display microcystin content per treatment group.

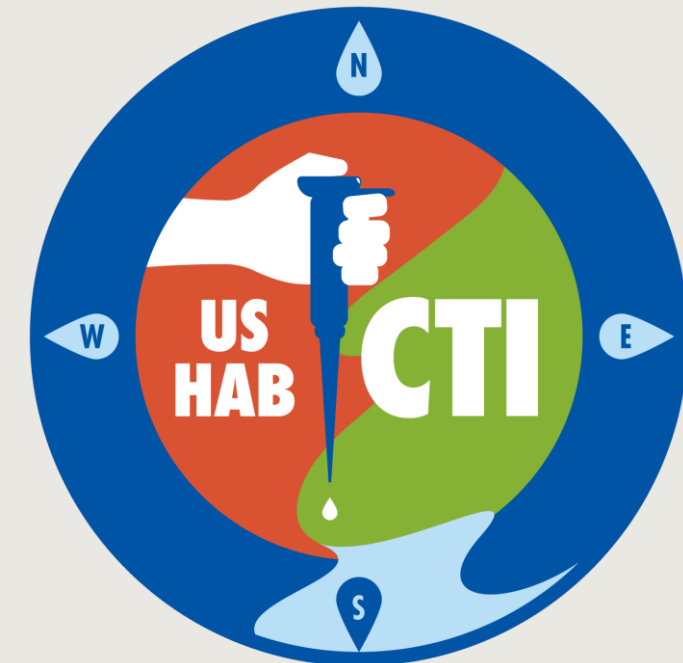
The results indicate that the treatments have varying degrees of success in reducing toxin production (**figure 5**). The combination of CaCl<sub>2</sub> and heat shock appears to have been the most effective, which does not align with our hypothesis. More exploratory work needs to be performed to determine the correct dosage of ASO and to improve the delivery of ASO into the cell.

## Conclusions and Challenges

- Hemocytometer is acceptable but imperfect for tracking cell counts. We are looking into acquiring a flow cytometer to improve cell counting methods
- Exploratory changes should be made to ASO assay procedure to determine most effective dosage and delivery system
- Differing methods of delivering heat shock may be more effective
- Encountered issues with the HPLC that required repairs to be made, resulting in a decrease in data collection.

## Acknowledgements

Thanks to Dr. Jayakody for the use of his centrifuge. Thanks to US HAB-CTI and NOAA for providing funding for this research. Thanks to the McNair scholar's program for providing additional funding and support for this research.





# Early life Stress Effects on Perineural Nets Within the Hippocampus-Amygdala Circuit

Daniyah Tate, Cladiua Ford, Michael Hylin, PhD  
Psychology, School of Human and Behavioral Sciences



## Introduction

“There is no effect with no cause, it doesn’t exist in this world. So, what is the cause and effect? These are children. These are children exhibiting what we call mimicry or learned behavior. They were not born this way, they are impressionable. Children are not always listening; I grant you that. But they are always learning no matter what and the small gesturers of adults the small unassuming conversations of parents are picked up by their children so there's an effects here but there's also a cause...” (Newus.88)

**Adverse Experiences in Early Life (ELS):** ELS encompasses emotional, sexual, and physical abuse as well as neglect (Bremner & Vermetten, 2001; Pechtel & Pizzagalli, 2011; Gould et al., 2012).

- Contribute to chronic stress in children, increasing their vulnerability to develop mental health disorders such as anxiety, depression, and substance abuse problems later in life (Weber et al., 2008; Green et al., 2010; Carr et al., 2013).
- Up to 45% of childhood-onset and over 30% of adult-onset mental health disorders are linked to ELS (Green et al., 2010; VanTieghem & Tottenham, 2017).
- ELS effects can be observed across generations in both humans and non-human primates (Greene et al., 2020; Zeynel & Uzer, 2020).

**Impact on brain development:** Includes the amygdala, hippocampus, and medial prefrontal cortex (mPFC) (LeDoux, 2000; Shin & Liberzon, 2010).

- Central role in emotional processing, learning, and memory (LeDoux, 2000; Shin & Liberzon, 2010).
- Disrupts development and function, leading to altered emotional responses, learning difficulties, and increased susceptibility to stress (Tottenham et al., 2011; Burghy et al., 2012; Gee & Casey, 2015).

**Perineuronal Nets (PNNs):**

- Specialized extracellular matrix structures surrounding neuronal cell bodies and proximal dendrites (Frischknecht et al., 2009).
- Composed of a meshwork of glycosaminoglycans and tenascin-R (TnR) molecules interacting with cell adhesion molecules (Brückner et al., 2003; Sorbonne et al., 2003).

**Functions of PNNs:**

- Creates a physical barrier that restricts the mobility of pre- and postsynaptic elements, promoting synaptic stability (Dityateva et al., 2008; Syková & Carulli, 2009).

**Synaptic Plasticity:**

- Regulates molecules involved in Long-Term Potentiation (LTP) and Long-Term Depression (LTD), essential for learning and memory (Pizzorusso et al., 2002; Weickert et al., 2011).

**Role in Development:**

**Critical Periods:** PNNs are involved in sculpting and refining neuronal circuits during critical developmental periods (Seki et al., 2002; Agnati et al., 2003).

Specific Effects of ELS on the Hippocampus and Amygdala:

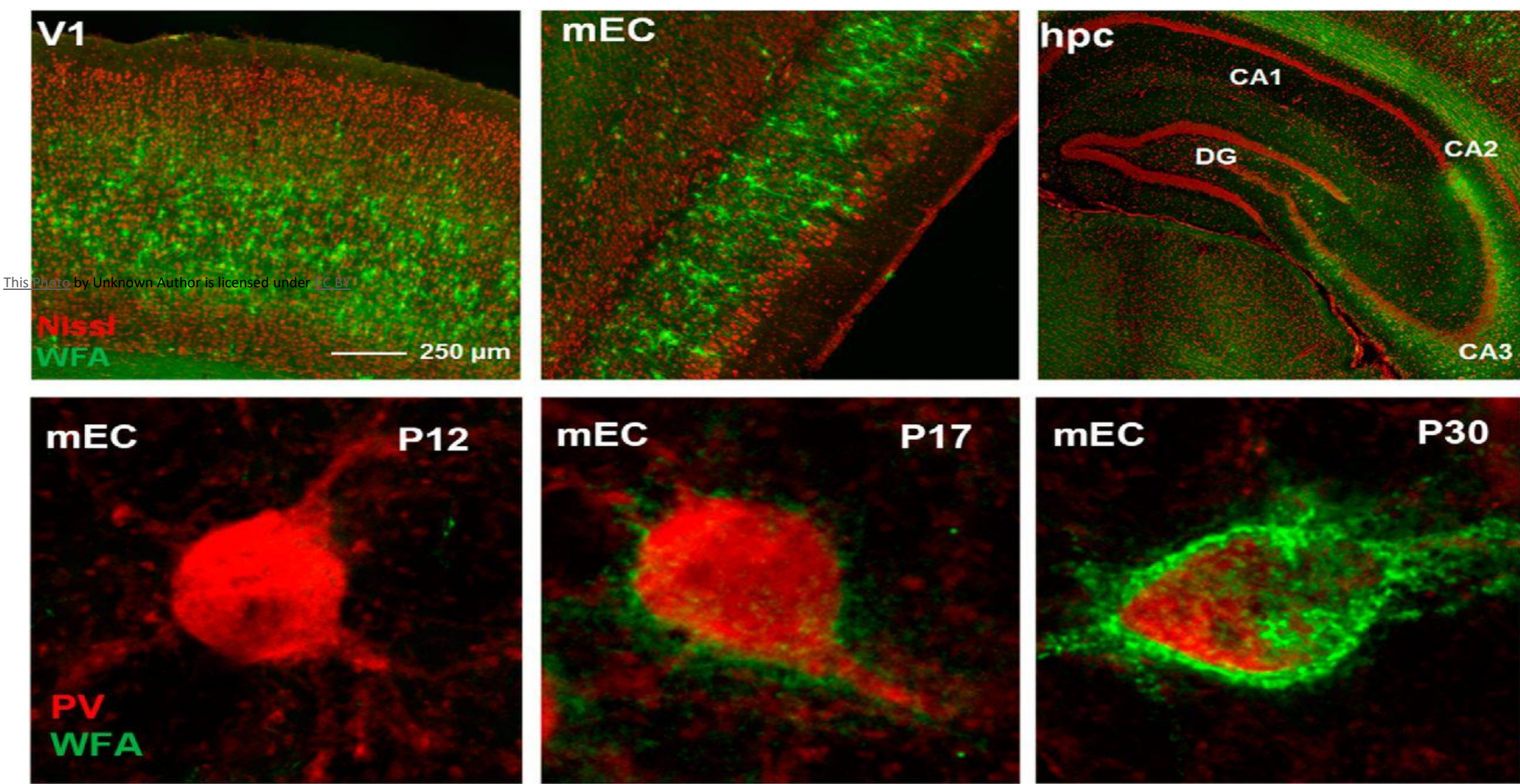
**Hippocampus:**

- Function:** Crucial for learning and memory.
- Impact of ELS:** Disrupts synaptic plasticity and stability, leading to cognitive deficits.

**Amygdala:**

- Function:** Key role in emotional processing.
- Impact of ELS:** Alters emotional responses and increases vulnerability to stress.

Understanding the impact of ELS on the development and function of PNNs around the hippocampus and amygdala can provide insights into the long-term consequences of early life stress on the brain. This knowledge may lead to new therapeutic interventions aimed at mitigating these effects.



## Method

This study builds upon existing research by Claudia Ford examining the effects of combined chronic early life stress (ELS) during adolescence. In this study we specifically focused on the perineural net (PNN) within the hippocampus and amygdala of these previously collected tissues.

**Tissue Source**

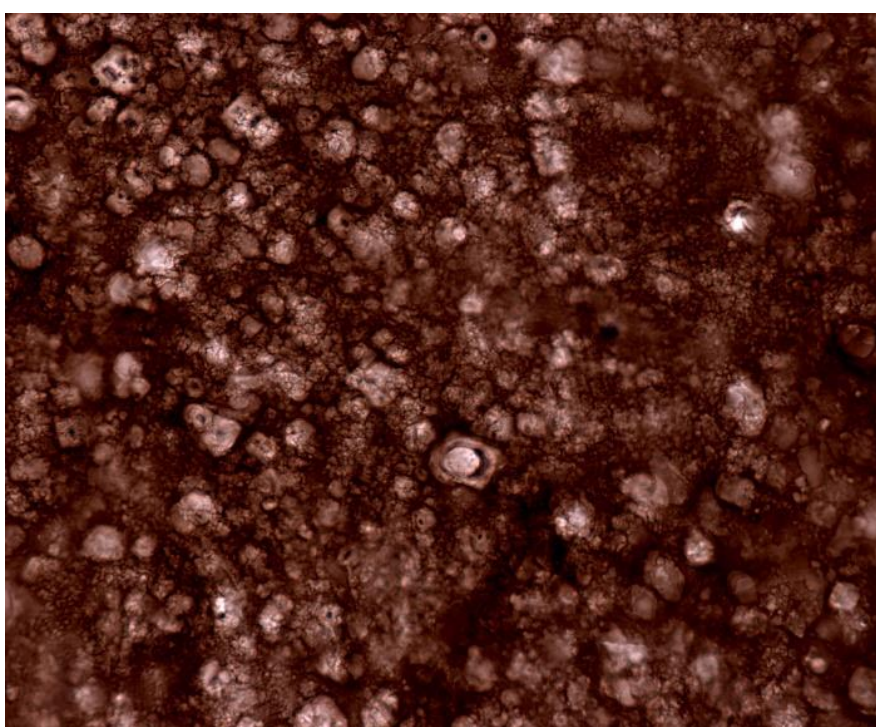
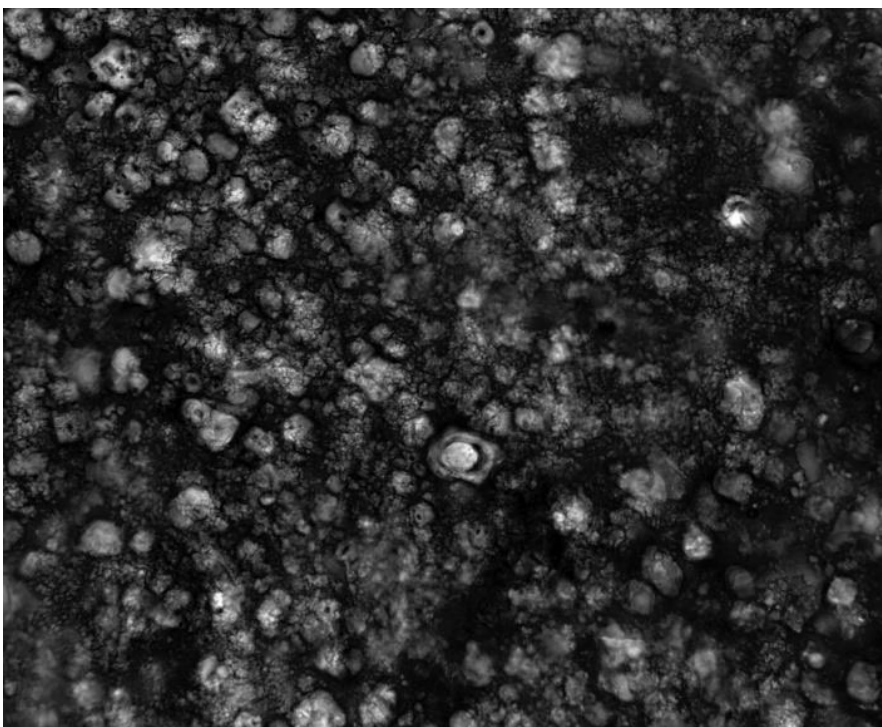
We archived brain tissues from the Fords study. These tissues were collected from male and female rats exposed to various ELS protocols:

- Control:** No exposure to ELS.
- Mild Stress:** limited bedding during the first two postnatal weeks.
- Moderate Stress:** Exposed to maternal separation
- Chronic Stress:** Combined experience of both mild and moderate stress protocols.

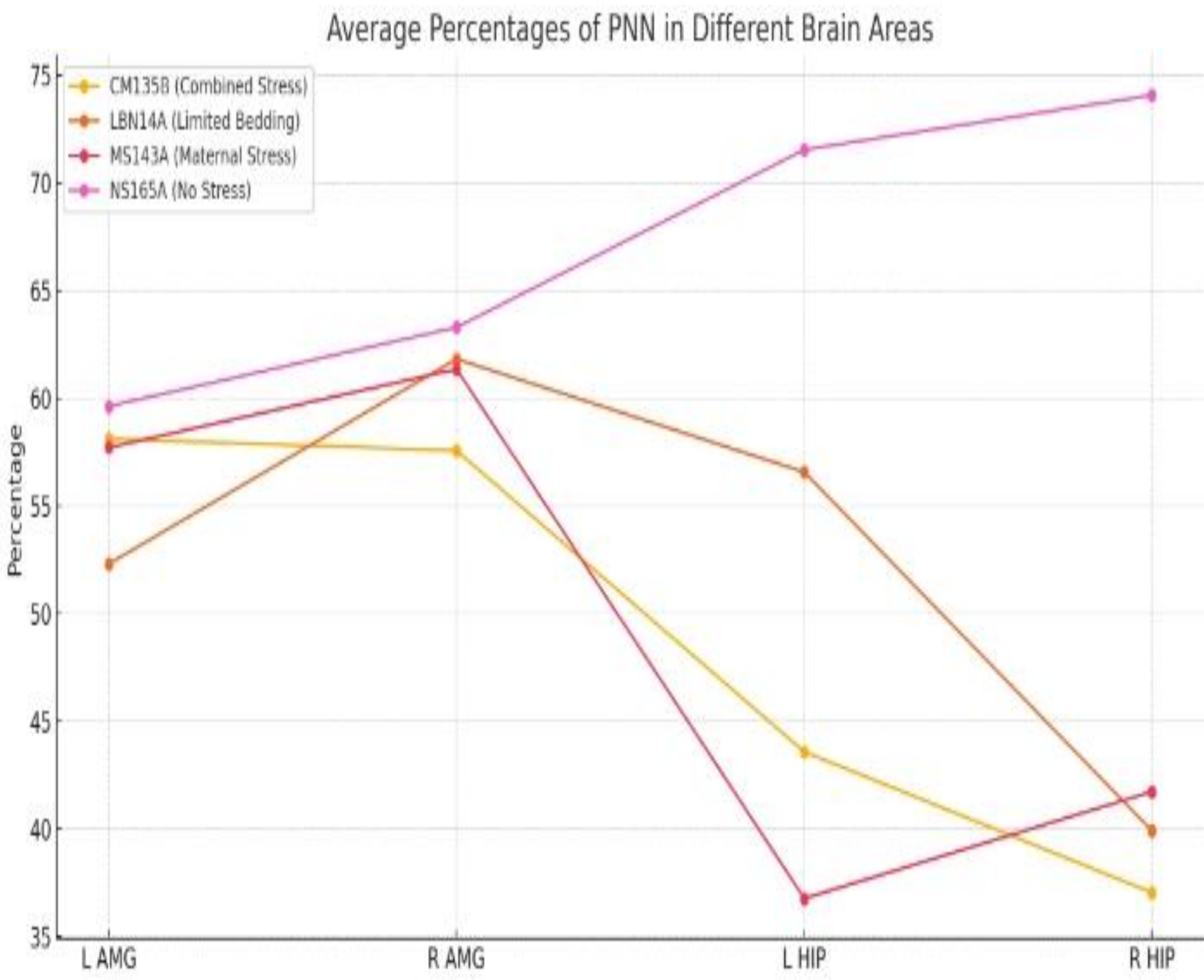
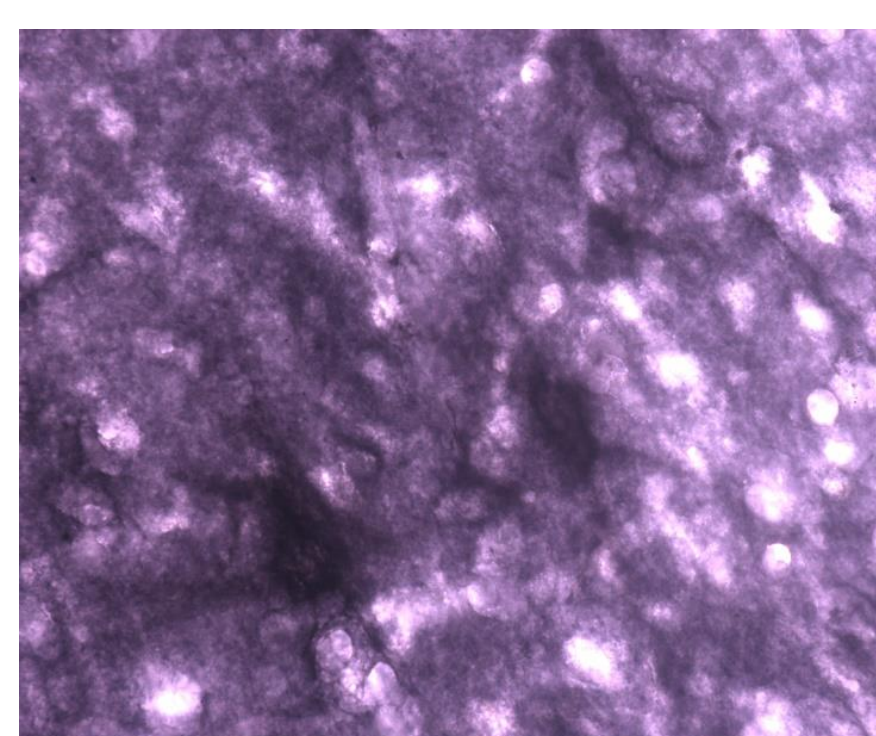
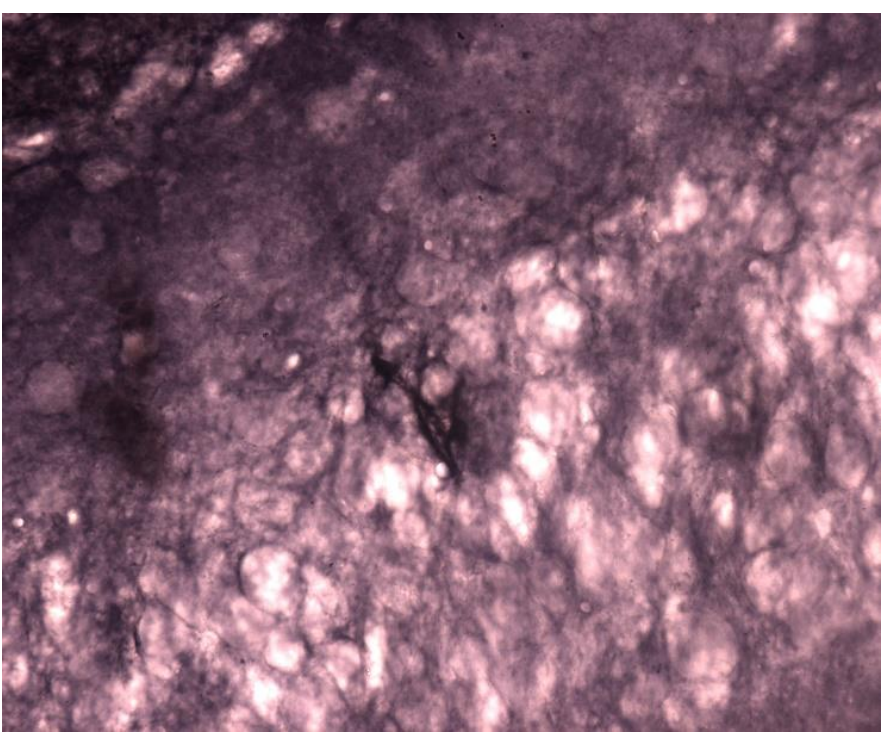
**PNN Analysis :** We focused on examining the PNN structure within the hippocampus and amygdala of these archived tissues. We used a microscope to visualize and quantify the PNN. This technique allows for assessment of parameters such as perineural sheath thickness in these areas. Since the tissues were collected at different time points in the original study by Ford, we compared PNN development across groups at each specific age point available. This approach allows us to investigate how ELS might influence the PNN's development over time.

## Result

Subject 165A- No Stress: Amygdala: Right Hemisphere



Subject 165A- No Stress: Amygdala: Right Hemisphere



## Results

It appears that early life stressors influence the development of perineuronal nets (PNN) in the hippocampus and amygdala differently:

**1.Combined Stress (CM135B)** shows higher PNN percentages in the hippocampus compared to the amygdala, particularly in the right hippocampus.

**2.Limited Bedding (LBN14A)** has a relatively balanced PNN presence in the left and right amygdala but shows a lower percentage in the right hippocampus.

**3.Maternal Stress (MS143A)** indicates higher PNN presence in the hippocampus, especially in the left hippocampus, suggesting significant impact from maternal stress.

**4.No Stress (NS165A)** has the highest PNN percentages in both the hippocampus and amygdala, indicating a higher baseline level of PNN without stress.

These observations suggest that stress can reduce PNN presence in the hippocampus and amygdala, with the effect varying based on the type and timing of the stress. The reductions are more pronounced in the hippocampus, especially with maternal and combined stress.

## Conclusion

**1.Impact of Early Life Stress on PNN Development:**

- Early life stress, whether mild (limited bedding), moderate (maternal separation), or chronic (combined stress), appears to reduce the presence of PNNs in the hippocampus and amygdala. This reduction is more pronounced in the hippocampus compared to the amygdala.
- The highest PNN percentages were observed in the no stress group (NS165A), indicating that normal development without stress leads to higher baseline levels of PNNs.

**2.Comparison of Different Stress Conditions:**

- Combined Stress (CM135B):** This group shows higher PNN percentages in the hippocampus compared to the amygdala, particularly in the right hippocampus. This suggests that chronic combined stress has a more detrimental impact on PNN development in the hippocampus.
- Limited Bedding (LBN14A):** This group exhibits a relatively balanced PNN presence in the left and right amygdala but lower percentages in the right hippocampus, suggesting that even mild stress can significantly impact hippocampal PNNs.
- Maternal Stress (MS143A):** Higher PNN presence in the left hippocampus indicates significant impact from maternal stress, aligning with findings that maternal separation during critical periods can disrupt normal brain development.
- No Stress (NS165A):** The highest PNN percentages across all brain areas suggest that a lack of stress supports optimal PNN development.

## Acknowledgment

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Thank You to McNair Scholars program for giving me the opportunity

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# The Impact of Socioeconomic Status (SES) on Language Development and Skills in Early Childhood – Meta Analysis

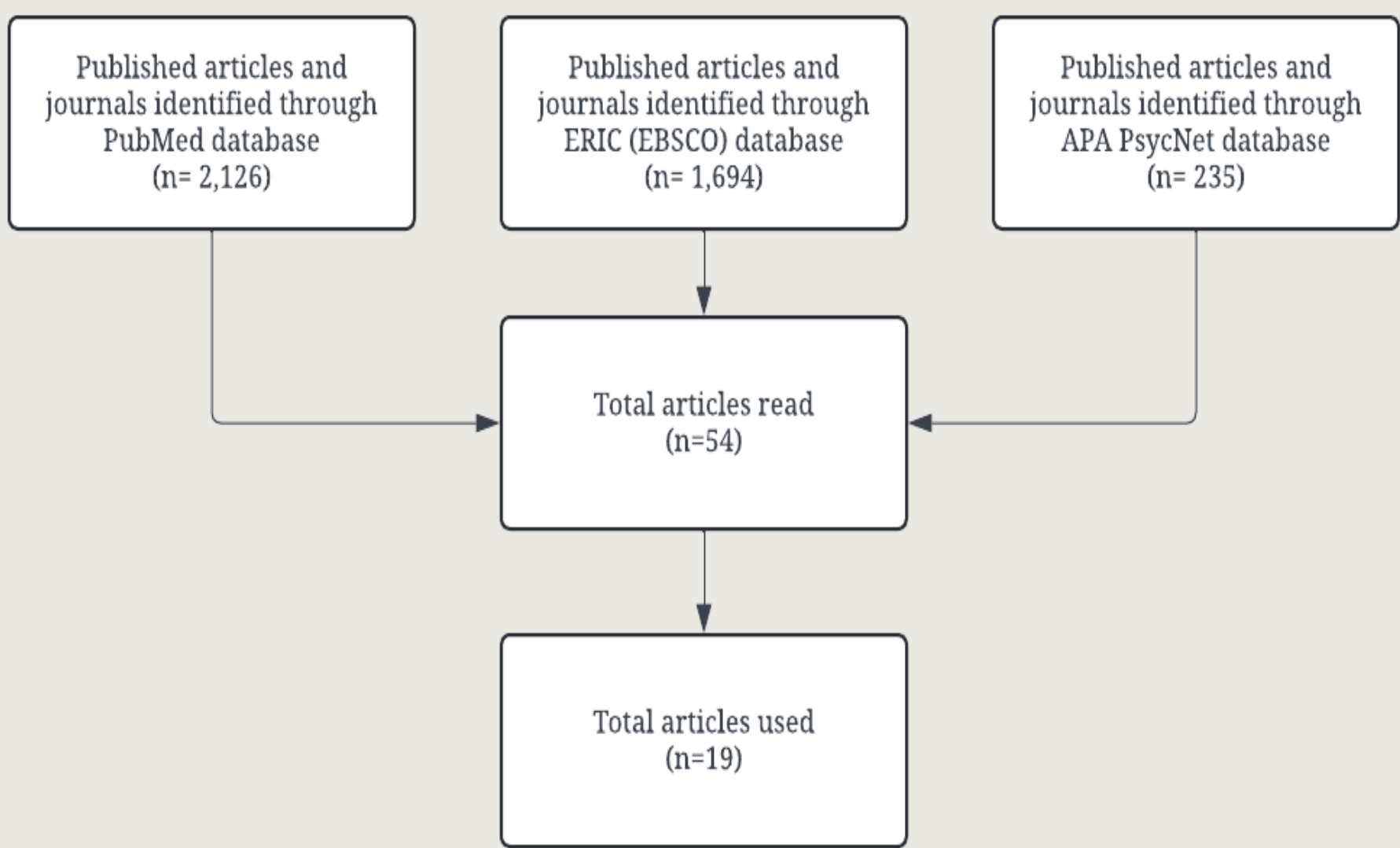
Melissa Valladares, Dr. Maria Claudia Franca  
Communication Sciences and Disorders



## Introduction

- Early childhood is a time for critical development, which includes the development of language. Increase in vocabulary, development of syntax, and language processing are just some of the many skills that children learn and develop.
- While all children develop language skills, rates of development vary from child to child. Many factors contribute to the varying rate, however socioeconomic status (SES) is one component that has a big impact. SES and its impact on language development and skills in early childhood has been widely researched.
- One of the most relevant studies was done by Hart and Risley in 1995.<sup>6</sup> They studied 42 families in Kansas from a wide range of SES. They found that children from higher SES families heard more words and a higher quality of word and gained more vocabulary words than those from lower SES families. They also estimated that by the time children are 4 years old, children from lower SES families hear 30 million less words than those from higher SES families.
- These findings indicate the importance of studying the effects of SES to get a more accurate picture.

## Methodology



- The studies that were included were done within the last 10 years (2014-2024), in English, and in the United States of America. Articles had a range of number of participants, ages, language assessments, SES and SES classifications
- Key words used were **language development or language acquisition or language learning** and **socioeconomic or social class or SES**. A second search was done using **language development or language acquisition or language learning** and **poverty or low income**.
- The databases used were PubMed, ERIC (EBSCO), and APA PsycNet. On PubMed there were 2,126 articles total with 8 relevant studies found, however only 5 were used after removing duplicates. On ERIC (EBSCO) there were 1,694 total articles found with 8 relevant articles used. On APA PsycNet there were 235 total articles found and 3 relevant and non duplicate studies were used.
- After going over the reference list for 11 articles 3 more articles were found bringing the total to 19 articles. Articles were excluded if the had a primary focus on bilingual children or if children in the study were not typically developing.
- Two of the articles found were published in 2013 and included, and one study done in 1995 was also included.

## Results

- When do differences in SES begin to impact language skills and development?
  - 7-month-old infants in the low SES group had lower scores on the PLS – 5.<sup>2</sup> While this study was done on a small sample, their findings were consistent with those done on older children.
  - A similar study found that SES differences were observable at 18 months. Children from higher SES families had **more increase in vocabulary** and were more **accurate in their comprehension**.<sup>4</sup>
- Does the effect of SES remain as children age?
  - The effects of SES remain as children enter preschool and kindergarten.<sup>6,10,13</sup>
  - Using the Quick Interactive Language Screener (QUILS) which assesses vocabulary, syntax, and language learning processes, studies have found that children from higher SES groups perform **significantly better** than those from lower SES groups.<sup>10,13</sup>
  - One significant finding from Levine et al. (2020), was that 5-year-old children from lower SES families were **1-2 years behind** their higher SES peers on all three QUILS components. Along with this, vocabulary scores for lower SES children were almost half of those from the higher SES group.
- What possible SES factors contribute to a difference in language development and skills in early childhood?
  - Higher parental education was associated with higher language skills scores.<sup>3,4,10</sup> It may account for more **advanced and diverse language** which activates areas in the brain related to language processing and learning.<sup>11</sup>
  - Parental stress was associated with dysregulated parent-child interactions which led to lower language skill scores<sup>8</sup>.
  - Household chaos also had a negative impact on children's language skills.<sup>9</sup>
  - Home literacy environment, such as **exposure to language** and activities for **engagement and support** of language development, had a positive impact on children. Children whose parents read to them more often or had access to books had **higher vocabulary skills**.<sup>3</sup>
  - Quality of communication, such as diverse language was found to be more impactful than the quantity of communication.<sup>7</sup>

- What intervention methods are there for closing or mediating the gap in language skills?
  - The effects of Duet, a community based participatory research project for low SES families focused on supporting caregivers and increasing developmental knowledge, self efficacy, quality of interactions, and children's language were studied. It was found to have a positive impact on all areas.<sup>1</sup>
  - Early Head Start Program positively impacts cognitive skills, vocabulary, health, and parents' engagement on children 2-3 years old.<sup>12</sup>
  - Supporting parents by providing and educating parents on resources has a positive impact on development. The use of social services **increases language skills** for infants in poverty.<sup>5</sup>
  - Providing **access to books** and educating on the importance of reading may also help.<sup>14</sup>

## Conclusion

- The purpose of this was meta-analysis was to examine the effects of SES on language development and skills, possible factors that contribute to these differences, and intervention methods for closing or mediating these gaps.
- These findings support Hart and Risley's findings that children from lower SES households have lower language skills than those from higher SES households.
- The studies reviewed found differences in vocabulary, syntax, language processing, and comprehension.
- Many of these studies had a diverse population sample which allows them to be applied to larger populations.
- Some limitations were that the populations were small, race was not included as a factor, and not using articles outside of the US.
- Some of the studies that focused on the effects of SES across different ages did not assess the same children for each age assessed. A longitudinal study

## Acknowledgements

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