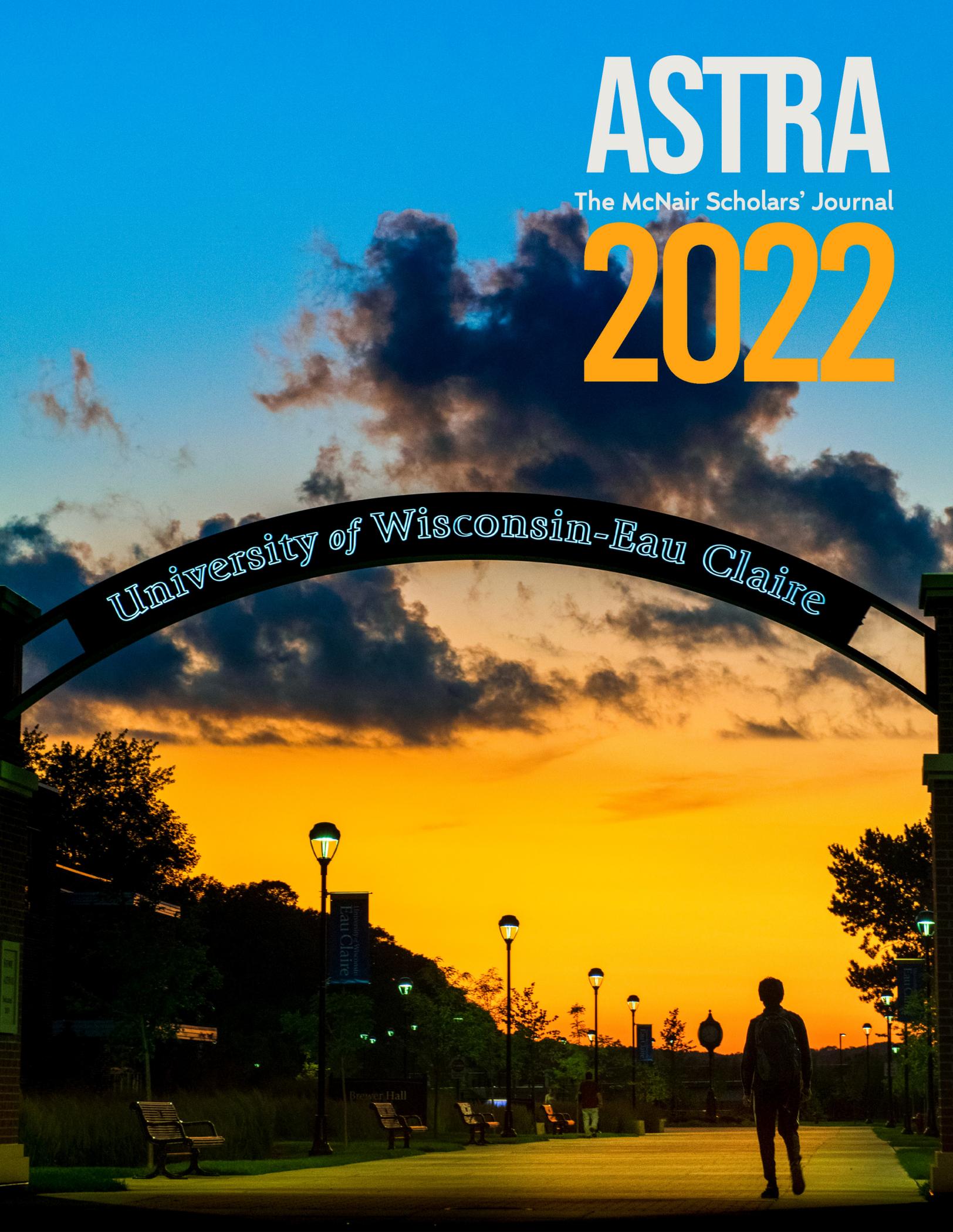


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The McNair Scholars' Journal

2022

University of Wisconsin-Eau Claire



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**Message from Provost and Vice Chancellor
Patricia A. Kleine**

COVID-19 has certainly continued to disrupt much of the University's "normalcy" during academic year 2021-2022. However, despite accommodations being made in response to the pandemic, undergraduate student-faculty research flourished.

Since 1988, UW-Eau Claire has been recognized as the University of Wisconsin System's Center of Excellence for Faculty and Undergraduate Student Research Collaboration. In 2016, UW-Eau Claire received the National Council on Undergraduate Research (NCUR) campus award for Undergraduate Research Accomplishments. In 2023, UW-Eau Claire will host the annual NCUR which will bring to campus 4,000-5,000 faculty and student researchers. Lastly, in the University's new 2025 strategic plan, one of only three goals is to reach an even higher level of national recognition for undergraduate research.

No program exemplifies the institution's commitment to opportunities in undergraduate research for students more than UW-Eau Claire's Ronald E. McNair Post-Baccalaureate Achievement Program. Because of the high academic quality of students' studies, McNair scholars have been named Fulbright, Goldwater, Truman, and Rhodes Scholars. This journal presents the culmination of two years of students working with their faculty mentors on critical questions in their disciplines and preparing their research for professional publication and presentation.

To the students, congratulations on the completion of your research projects, and best wishes for continued success in graduate school.

To my faculty colleagues, thank you for mentoring these remarkable students so well. I continue to celebrate your exceptional commitment to student success.

To the reader, I hope you enjoy reviewing this journal and the wealth and breadth of research within it.

Patricia A. Kleine
Provost and Vice Chancellor for Academic Affairs
Academic Year 2021-2022

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- Dr. James Phillips, Department of Chemistry and Biochemistry
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- Dr. Thao Yang, Department of Chemistry and Biochemistry

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Do atropine and diphenhydramine, two anti-cholinergic drugs, interact to affect heart rate?

**Savanna Bonlender
Dr. Tali Lee**

Abstract

Pharmaceuticals are often prescribed together to treat varying symptoms, so understanding how drugs interact mechanistically is important for guiding a safe and effective treatment plan. Two common drugs, atropine and diphenhydramine are both classified as anti-cholinergic, which means they block the action of acetylcholine resulting in an increase in heart rate. However, these drugs are prescribed for different ailments, so they may be administered concurrently, which poses the risk for unintended side effects. The goal of this study was to investigate atropine and diphenhydramine both independently and in combination by measuring their effects on heart rate using the model organism *Daphnia magna*. Atropine increased heart rate by 17% compared to control, while diphenhydramine increased heart rate by 27% compared to control. In combination, heart rate increased by 30%, which was similar in magnitude to the effect seen by diphenhydramine alone. In a second trial, diphenhydramine increased heart rate even in the presence of a drug that reduces its ability to bind to one of its receptors suggesting its mode of action is more complicated. However, because heart rate increased similarly when drugs were administered independently or in combination, evidence does not suggest the two drugs interact to adversely affect heart rate.

Introduction

A pharmaceutical is a substance that is used to treat an illness or alleviate symptoms by interacting with specific target molecules. Depending on the active ingredient, pharmaceuticals work by targeting various enzymes, receptors, transporters, and ion channels found within the body to induce a physiological effect. By utilizing these mechanisms, pharmaceuticals can work to target a specific region or organ within the body, but because the certain receptors or channels with which they interact are often found throughout the body, pharmaceuticals may work on other regions of the body, which may result in unintended side effects. Furthermore, multiple pharmaceuticals are often prescribed together. In these cases, their effects may be independent of each other, or one of three primary types of interactions can occur. An additive interaction is when the effect of two chemicals is equal to the sum of the effect of the two chemicals taken separately, whereas a synergistic interaction means that the effect of two chemicals taken together is greater than the sum of their separate effect at the same doses. An antagonistic interaction means that the effect of the two chemicals is less than the sum of the effect of the two drugs taken independently of each other (Yeh et al., 2007). Understanding how two or more

drugs interact is important to guide effective and safe treatments.

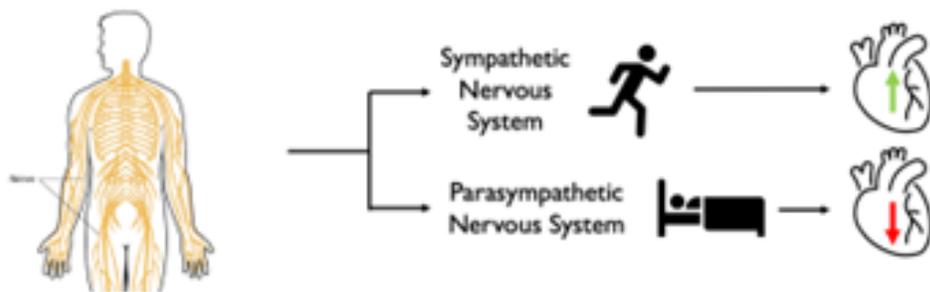


Figure 1. Visual representation of the two branches of the autonomic nervous system and how they affect heart rate



Figure 2. Anatomical structure of the *Daphnia magna*

One of the most common classifications of drugs are parasympathetic nervous system agents. The parasympathetic nervous system is a branch of the peripheral nervous system within the autonomic nervous system (Fig. 1). The parasympathetic nervous system is responsible for stimulating digestion and initiating relaxation throughout the body. Organs receive parasympathetic innervation by receiving messages from various nerves. A common nerve that is associated with parasympathetic innervation is the vagus nerve. Specifically, the heart is an organ that is innervated by parasympathetic cholinergic nerves that are derivatives of the vagus nerve. Here, acetylcholine is released by these nerve fibers and binds to the M2 muscarinic receptors (Bardal et al., 2011). In the cardiac muscle tissue, the primary regions that are under innervation from the vagus nerve are the sinoatrial (the pacemaker) and atrioventricular nodes. When stimulated, the vagus nerve produces a negative chronotropic effect, resulting in decrease in heart rate.

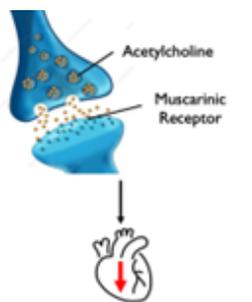


Figure 3. Visual representation of acetylcholine's mechanism to initiate a decrease in heart rate.

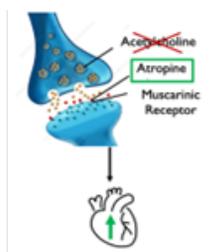


Figure 4. Visual representation of Atropine (ATR) competing for the muscarinic receptor by blocking the binding of acetylcholine, resulting in an increase in heart rate.

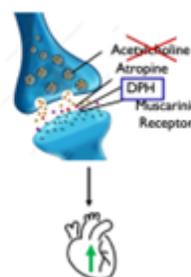


Figure 5. Visual representation of diphenhydramine (DPH) and atropine competing for muscarinic receptors blocking the binding of acetylcholine.

Daphnia magna are a type of water flea that serve as beneficial testing organisms because they are cost efficient, easy to handle and manipulate, and have similar cellular receptors as humans (Fig. 2) (Tkaczyk et al., 2021). *Daphnia* are the oldest and most utilized testing organisms for various aspects of biological research (Tkaczyk et al., 2021). Throughout the past ten years, the use of *Daphnia* as a model testing organism has become more popular in pharmacological research because of their sensitivity to chemicals. It has been found that the *Daphnia* heart is partially innervated by the vagus nerve, which is the same nerve that innervates the human heart

(Bekker et al., 1951). Therefore, *Daphnia* may be ideal organisms to test questions regarding drugs that work through the vagus nerve or by similar mechanisms to initiate their effects.

Acetylcholine is the chemical messenger that allows the neural impulse from the vagus nerve to communicate with the muscular tissue to initiate its effects, therefore it acts as a neurotransmitter. Once acetylcholine is released, it binds to muscarinic receptors within the heart, which then initiates a signal within the heart muscle tissue leading to contraction of smooth muscles, dilation of blood vessels, and bradycardia (decreased heart rate) (Fig. 3) (Bekker et al., 1951).

Atropine is an anticholinergic chemical that has many clinical uses. Primarily, atropine is used to reduce bronchiole secretions, but it is also used to treat bradycardia (low heart rate). As it pertains to this project, atropine works as a muscarinic receptor antagonist, meaning it blocks the action of acetylcholine therefore working to inhibit vagus nerve stimulation (Fig. 4) (Carvalho et al., 2003). Since the vagus nerve works through the parasympathetic nervous system to slow heart rate, atropine works to counter the effects of the parasympathetic nervous system to increase heart rate (Lomba et al., 2020). There is little research published on the effects of atropine on the *Daphnia* heart rate, and within these experiments, there appears to be contradictory results of the effect of atropine on the *Daphnia* heart rate (Baylor et al., 1942) (Bekker et al., 1951) (Villegas-Navarro 2003).

Diphenhydramine is classified as both a first generation H1-antihistamine and an anticholinergic drug that also works by targeting muscarinic-3 receptors (Fig. 5) (Sicari & Zabbo, 2020). Diphenhydramine, the primary ingredient in Benadryl[®], is most utilized for its antihistamine properties (Kristofco et al., 2014) as it also blocks histamine receptors, therefore it is able to be utilized for many other conditions such as motion sickness, difficulties sleeping, and can even help manage symptoms of Parkinson's disease. Since there are widespread bodily effects from diphenhydramine, there is an increased possibility for an interaction with other anticholinergic drugs to occur. In a case report conducted by Abdi et al. (2014) it was found that diphenhydramine toxicity presents with symptoms such as tachycardia, encephalopathy, and dry mucous membranes. These symptoms are due to the anticholinergic nature of diphenhydramine.

The objective of my study was to investigate atropine and diphenhydramine both independently and in combination by measuring their effects on heart rate using the model organism *Daphnia magna*. Given that diphenhydramine and atropine are both anticholinergic, I hypothesized they would both increase heart rate independently and when combining these drugs, a synergistic interaction will occur, meaning the increase in heart rate caused by these two drugs concurrently will be greater than when taking the two drugs separately.

Materials/Methods

First, an atropine dose-response was conducted. Using preliminary trials, it was found that concentrations below 10^{-8} M atropine did not initiate a response, so 10^{-8} M was chosen to be the most dilute concentration. Dilutions were made starting with 10^{-2} M atropine and were diluted by one order of magnitude until 10^{-8} M atropine (10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, 10^{-4} M). Next, a diphenhydramine dose-response was similarly conducted with diphenhydramine at the following concentrations: 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M, and 10^{-4} M.

Following the independent dose-response trials, *Daphnia* were treated to combinations of atropine and diphenhydramine to evaluate potential interactions. First, 10^{-8} M diphenhydramine was administered to the *Daphnia* as a background with atropine increasing in concentrations

by one order of magnitude (10^{-8} M- 10^{-5} M atropine) at each step resulting in the following dose-response trial concentrations: 10^{-8} M diphenhydramine/ 10^{-8} M atropine, 10^{-8} M diphenhydramine/ 10^{-7} M atropine, 10^{-8} M diphenhydramine/ 10^{-6} M atropine, and 10^{-8} M diphenhydramine/ 10^{-5} M atropine.

Since diphenhydramine has two drug classifications, a third experiment was conducted to expose the role of the histamine receptors in diphenhydramine's mechanism of action. To do this, carbachol, an acetylcholine mimic, was added to compete with diphenhydramine. This limits its ability to bind to muscarinic receptors, thus exposing the magnitude of response from diphenhydramine binding to histamine receptors alone. To begin, a carbachol dose response was conducted at 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-5} M and 10^{-4} M. The dose response trial revealed that 10^{-7} M carbachol initiated the greatest effect, so in the interaction trial, 10^{-7} M carbachol was held constant as the background with increasing concentrations of diphenhydramine, yielding the following experimental treatment solutions: 10^{-7} M carbachol/ 10^{-10} M diphenhydramine, 10^{-7} M carbachol/ 10^{-8} M diphenhydramine, 10^{-7} M carbachol/ 10^{-6} M diphenhydramine, and 10^{-7} M carbachol/ 10^{-6} M diphenhydramine.

Using preliminary time trials, it was determined the adequate exposure times for each drug (Table 1). Each *Daphnia* were exposed to 100 μ L of the treatment solutions in increasing concentrations with pond water as the control. Since the *Daphnia* heart beats too rapidly at room temperature to accurately visualize the heart under a microscope, the *Daphnia* were chilled to a constant 10 degrees Celsius while obtaining measurements. Upon exposing the *Daphnia* to the solution, heart rate was obtained by counting the number of times the heart physically beat by visualizing the heart through the microscope in the designated amount of time determined by preliminary trials. Data were analyzed by Repeated Measures ANOVA with Tukey HSD post-hoc tests (JMP software, SAS Institute Inc.).

Table 1. Adequate exposure time for drugs determined by preliminary time trials

Drug	Exposure Time (seconds)
Atropine	60
Diphenhydramine	120
Atropine + Diphenhydramine	120
Carbachol	120
Carbachol + Diphenhydramine	120

Results

Atropine

When exposed to increasing concentrations of atropine, *Daphnia* heart rate increased significantly (Fig. 6). On average, heart rate increased by 17% compared to control ($F = 3.32$, $P = 0.0041$). The 10^{-7} M concentration of atropine was found to be the threshold dose response. In an attempt to discover whether the effects of this drug are reversible, *Daphnia* were re-exposed to the control solution, pond water. This effect appeared to be reversible as heart rate declined during the washout recovery.

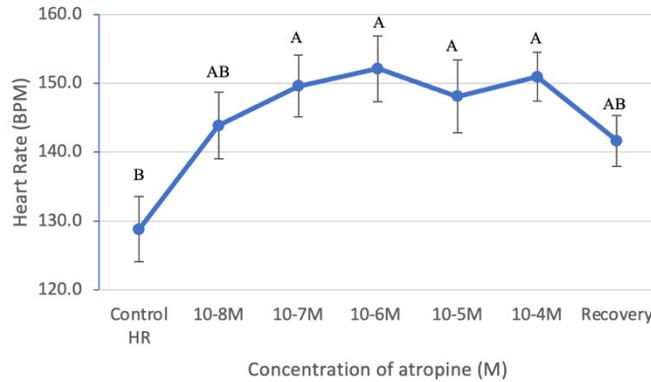


Figure 6. Mean heart rate (beats per minute) of *Daphnia magna* treated with increasing atropine concentrations (pond water, 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). *Daphnia* were maintained at 10°C and exposed to each concentration of atropine for 60 seconds before counting heart beats. Each point represents the mean heart rate of 25 *Daphnia* ($F=3.32$, $P=0.0041$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Diphenhydramine

When exposed to increasing concentrations of diphenhydramine, *Daphnia* heart rate increased significantly (Fig. 7, $F= 11.097$, $P= <0.001$). On average, heart rate increased by 27% compared to control. Using preliminary dose response trials (data not shown), it was determined that 10^{-8} M was the threshold concentration for diphenhydramine. The increase in heart rate in response to diphenhydramine was reversible.

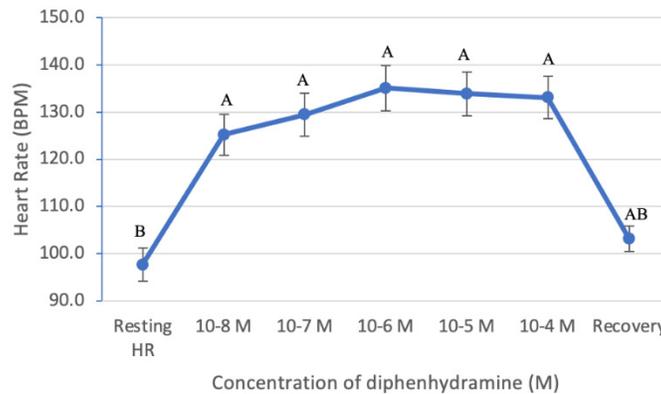


Figure 7. Mean heart rate (beats per minute) of *Daphnia* treated with increasing diphenhydramine concentrations (pond water, 10^{-8} , 10^{-7} , 10^{-6} , 10^{-5} , and 10^{-4} M). *Daphnia* were exposed to each concentration of diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=11.097$, $P<0.001$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Atropine and Diphenhydramine in Combination

When exposed to increasing concentrations of atropine and diphenhydramine, heart rate significantly increased. On average, heart rate increased by 30% compared to control (Fig. 8, $F= 26.67$, $P<0.001$). Atropine was exposed to the *Daphnia* increasing in concentration by two orders of magnitude, while diphenhydramine was in the background. It was found that atropine and diphenhydramine increased heart rate, but the magnitude of response was similar in magnitude to diphenhydramine alone.

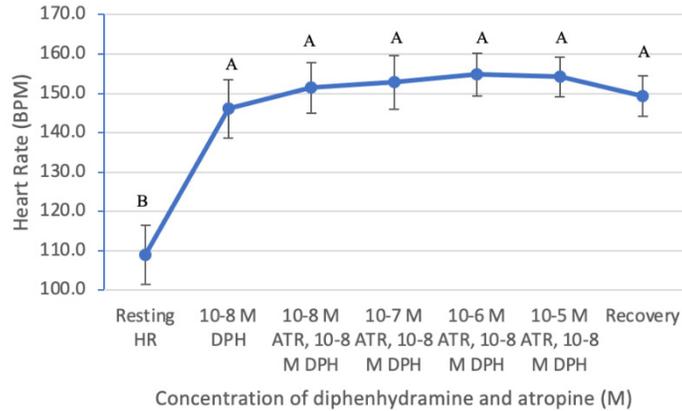


Figure 8. Mean heart rate (beats per minute) of *Daphnia* treated with increasing concentrations of atropine (ATR, 10⁻⁸-10⁻⁵ M) against a constant 10⁻⁸ M diphenhydramine (DPH) background. *Daphnia* were exposed to each concentration of diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* (F=26.67, P<0.001). Error bars represent the standard error. Means with different letters are significantly different at p<0.05 (Tukey HSD).

Carbachol

When exposed to increasing concentrations of carbachol, an acetylcholine mimic, heart rate significantly decreased (Fig. 9, F= 19.28, P<0.001). On average, heart rate decreased by 28% compared to control (Fig. 8, F= 19.28, P<0.001). Carbachol at 10⁻⁷ M was found to be the threshold dose response to initiate a decrease in heart rate, while 10⁻⁵ M exhibited the greatest decrease in heart rate. This confirms carbachol decreases heart rate as would be expected by acetylcholine in *Daphnia*.

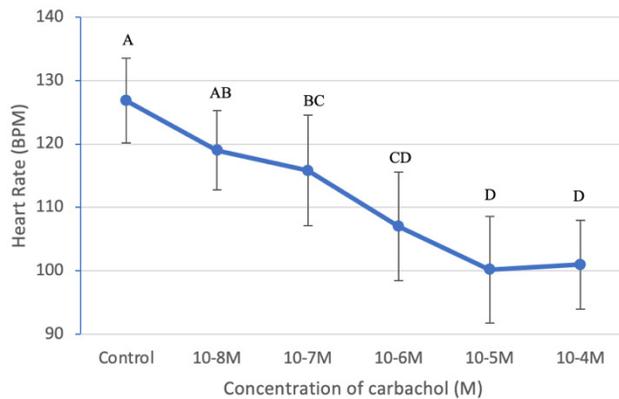


Figure 9. Mean heart rate (beats per minute) of *Daphnia* treated with increasing carbachol concentrations (pond water, 10⁻⁸, 10⁻⁷, 10⁻⁶, 10⁻⁵, and 10⁻⁴ M). *Daphnia* were exposed to each concentration of carbachol for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* (F=19.28, P<0.001). Error bars represent the standard error. Means with different letters are significantly different at p<0.05.

Carbachol and Diphenhydramine in combination

When exposed to increasing concentrations of diphenhydramine with carbachol at a constant background concentration of 10⁻⁷ M, *Daphnia* heart rate significantly increased. On average, heart rate increased by 14% compared to control (Fig. 10, F=12.75, P<0.01). The presence of carbachol reduced the effect of diphenhydramine by approximately 50% compared to the effect

of diphenhydramine alone (Fig. 7).

Carbachol and Diphenhydramine in combination

When exposed to increasing concentrations of diphenhydramine with carbachol at a constant background concentration of 10^{-7} M, *Daphnia* heart rate significantly increased. On average, heart rate increased by 14% compared to control (Fig. 10, $F=12.75$, $P<0.01$). The presence of carbachol reduced the effect of diphenhydramine by approximately 50% compared to the effect of diphenhydramine alone (Fig. 7).

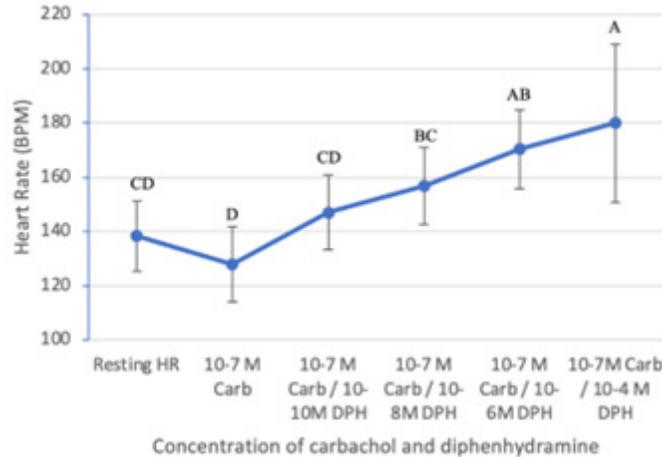


Figure 10. Mean heart rate (beats per minute) of *Daphnia* treated with increasing concentrations of diphenhydramine combined with a constant carbachol concentration (pond water, 10^{-7} M carbachol, 10^{-7} M carbachol/ 10^{-10} M diphenhydramine, 10^{-7} M carbachol/ 10^{-8} M diphenhydramine, 10^{-7} M carbachol/ 10^{-6} M diphenhydramine, and 10^{-7} M carbachol/ 10^{-4} M diphenhydramine). *Daphnia* were exposed to each concentration of carbachol and diphenhydramine for 120 seconds before counting heart beats. Each point represents the mean heart rate of 15 *Daphnia magna* ($F=12.75$, $P<0.01$). Error bars represent the standard error. Means with different letters are significantly different at $p<0.05$ (Tukey HSD).

Discussion/Conclusion

Atropine and diphenhydramine, two-anticholinergic drugs, were exposed to the *Daphnia magna* both independently and in combination to shed light upon the potential interaction between drugs with a similar mechanism of action. Both atropine and diphenhydramine increased *Daphnia* heart rate independently, and when applied in combination, their effects were similar in magnitude to when exposed to diphenhydramine alone. In addition, this study evaluated whether *Daphnia magna* are prime test subjects for anti-cholinergic drug testing. Because the *Daphnia* are easy to maintain, cost efficient, and responded in a similar manner to humans, it was determined *Daphnia* are prime test subjects for anti-cholinergic drug testing.

Since the pharmaceutical industry is rapidly developing and is in need of cost-efficient testing organisms (Tkaczyk et al., 2021), it was pertinent to discover if *Daphnia* are under innervation of a nerve similar to the vagus nerve, since that is the mechanism of action in the parasympathetic nervous system of humans. Evidence from this study supports the hypothesis that *Daphnia* have a nerve similar to the vagus nerve because the *Daphnia magna* responded to atropine, diphenhydramine, and carbachol similar to the response observed in humans.

This current study showed that atropine significantly increased heart rate when exposed to increasing concentrations of atropine similar to findings from other studies (Villegas-Navarro 2003). However, these results contradict the findings in Bekker et al., (1951). Therefore, this supports the hypothesis that atropine blocks the deaccelerating effects of the parasympathetic nervous system, resulting in an increase in heart rate. Similarly, when *Daphnia* were exposed to increasing concentrations of diphenhydramine, heart rate increased significantly. These data support the hypothesis that atropine and diphenhydramine, via their anti-cholinergic properties, both independently increase heart rate.

Although atropine and diphenhydramine are both classified as anti-cholinergic drugs and pose an interaction risk if consumed together (Yeh et al., 2007), evidence supports that these drugs in combination, do not interact. When *Daphnia* were exposed to the drugs in combination, the impact was similar in magnitude to when exposed to diphenhydramine alone. These data answered the question of whether an interaction occurs, but it is unclear as to why diphenhydramine independently increased heart rate 10% more than atropine.

There are likely several possible explanations as to why diphenhydramine caused a greater increase in the heart rate of *Daphnia* than atropine. First, it is possible that diphenhydramine has a greater affinity for the muscarinic receptors compared to atropine, meaning the molecules more readily bind to the muscarinic receptors, thus initiating a greater response. To test this hypothesis, the chemical kinetics would need to be evaluated. This would include obtaining the chemical dissociation constant for atropine binding to the muscarinic receptor. Understanding the kinetics would best inform how readily these molecules interact with their receptors.

The second explanation as to why diphenhydramine initiated a greater response may be related to its antihistamine properties. While diphenhydramine interacts with muscarinic receptors, it also binds histamine receptors that may also lead to an increase in heart rate via a mechanism independent of its action as an acetylcholine antagonist. This study found that when carbachol, an acetylcholine mimic, was added in combination to diphenhydramine, *Daphnia* heart rate still increased by 12%, which is a lesser extent than when exposed to diphenhydramine alone.

Even in the presence of a drug that reduces the ability of diphenhydramine to bind to muscarinic receptors as part of the parasympathetic pathway, diphenhydramine was still able to increase heart rate. Therefore, histamine receptors likely have a role in the response. The extent of the involvement of the histamine receptors in the response of diphenhydramine, in addition to the affinity of diphenhydramine for the histamine and muscarinic receptors remain unknown. Berninger et al., (2011) found that diphenhydramine initiated a greater response in *Daphnia magna* compared to other aquatic organisms, but the reason why this occurred is unknown. There is little research investigating the effects of antihistamines on the *Daphnia magna* heart rate, thus future studies could investigate the kinetics of these reactions. Also, additional information about the role of diphenhydramine binding to the histamine receptors in the acceleration response in *Daphnia* is needed and would inform our understanding of how atropine and diphenhydramine

should best be used to guide safe and effective treatments in humans.

Acknowledgements

Thank you to the UWEC diversity mentoring program, UWEC Ronald E. McNair program, Dr. Kelly Wonder, Dr. Ka Vang, and Dr. Brad Carter for their support and guidance through this project. Thank you to my mentor, Dr. Tali Lee, for her mentorship, diligence, and dedication.

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Studies of 4-Amino-¹H-pyrazolo[3,4-d]pyrimidine-6-ol (4APPY6O) Binding to Xanthine Oxidase Modeled After Urate Binding

Keng Chang
Dr. Thao Yang

Abstract

This is an article on the binding results of 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol binding to xanthine oxidase (XOD). XOD is an enzyme found in the human serum and lungs. The disease gout is caused by formation of high level of uric acid in the blood. Molecules that can bind to XOD are potential inhibitors, which could be developed as possible remedies for treating gout symptoms. The compound consists of the pyrazolopyrimidine skeletal structure with different peripheral groups around the fused rings compared to uric acid, a natural final ligand. The binding studies were first carried out by computer docking of the molecule onto the XOD active site, then the results of interactions between the docked molecule and side chain groups, as well as the affinity energies, were evaluated compared to the known binding of uric acid and allopurinol to XOD. The computer docking results showed that the molecule formed similar hydrogen bonding pattern and hydrophobic interactions at the active side when compared to the results obtained by docking uric acid and allopurinol. This work was carried out by former colleague Eric Colwitz. The actual binding of 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol to XOD was carried out in solution by the technique Saturation Transferred Difference NMR spectroscopy (STD-NMR) by the current author (Mr. Keng Chang). The binding results obtained from the STD-NMR experiments showed that 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol have interactions with XOD. The amplification factor (A_{STD}) for the various ratios of ligand:protein was inconclusive at this time.

Introduction

Xanthine Oxidase (XOD) is an enzyme that converts hypoxanthine to xanthine then to uric acid (salt form, sodium urate), a metabolic waste product, Figure 1 (Fig. 1) (1). The XOD structure contains two subunits, each containing a molybdopterin group and one flavin adenine dinucleotide group (FAD) (2). When the activity of XOD is accelerated the level of uric acid increases and high level of this can lead to a buildup of urate crystals in a person's joints, leading to the cause of gout disease.

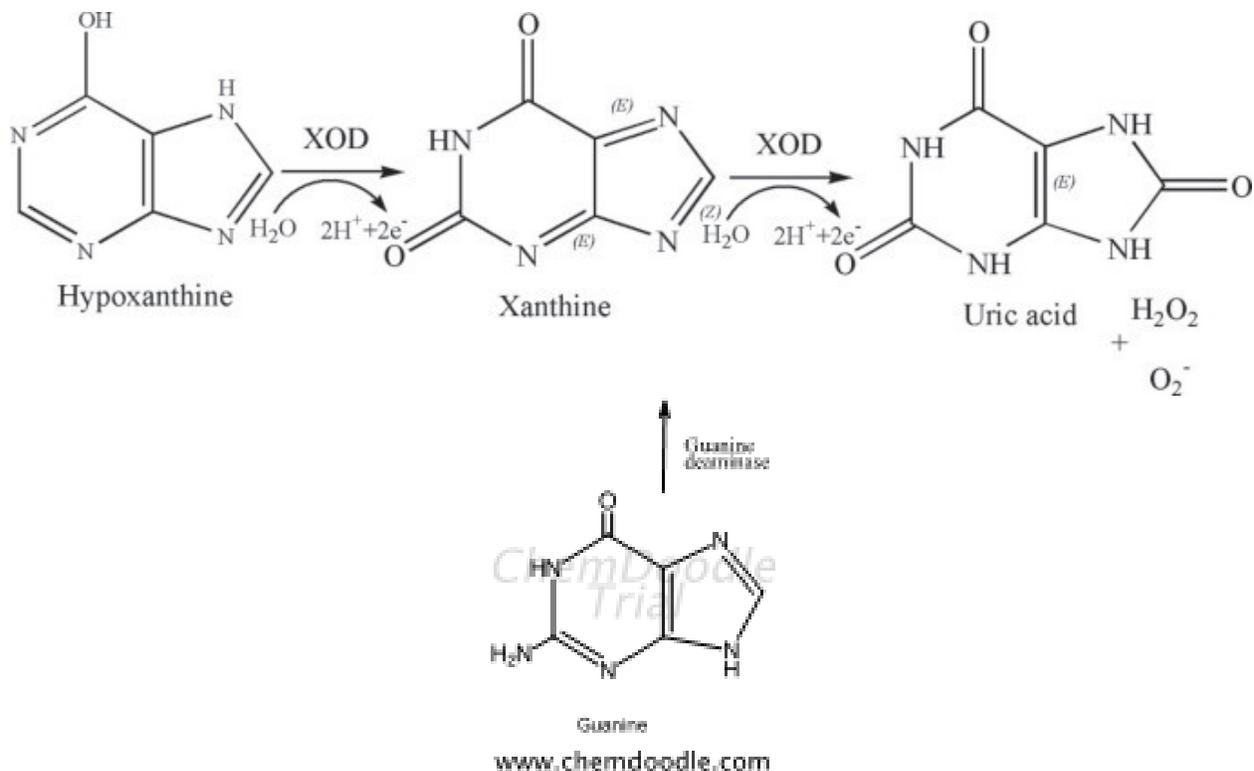
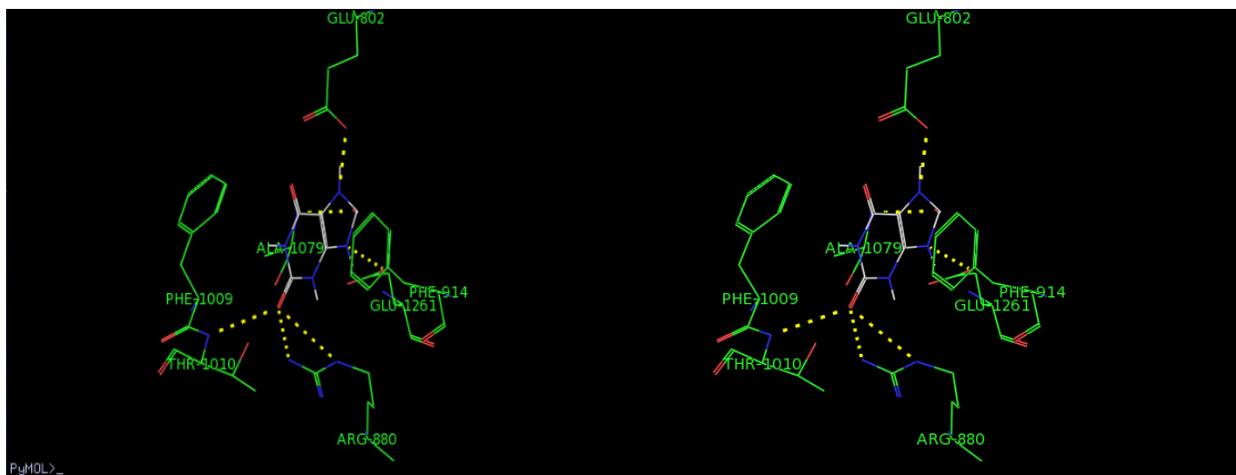


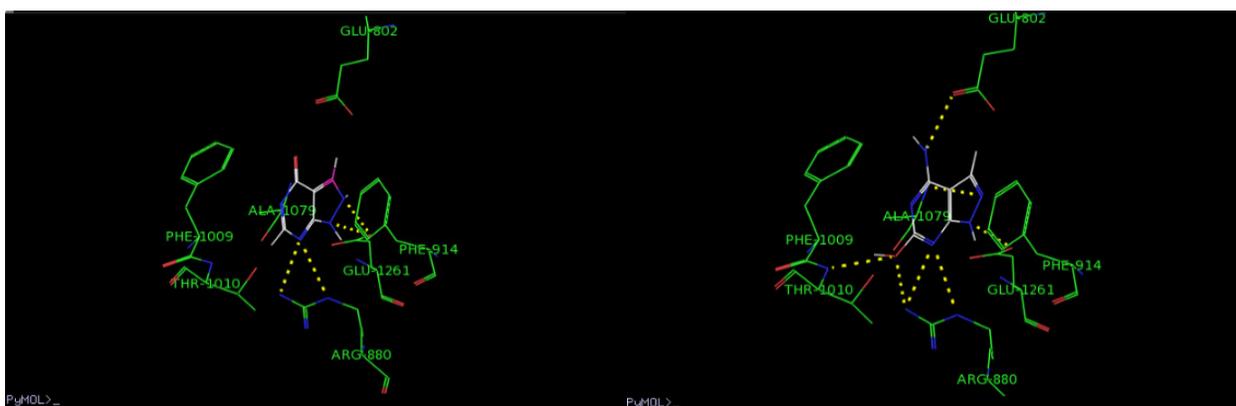
Figure 1. Oxidation of adenine to hypoxanthine, xanthine and guanine to produce uric acid

In this study, we investigate the binding of a small molecule, 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol (4APPY6O), to XOD by computational ligand-docking method using the Autodock Vina software (3, 4), and the actual binding of 4APPY6O to XOD in solution by Saturation Difference Nuclear Magnetic Resonance Spectroscopy (STD-NMR) (5, 6). The docking of uric acid binding to XOD was first performed and compared the results to the x-ray crystal structure of XOD-urate bound structure. Then, the same docking (uric acid to XOD) was compared to the docking of allopurinol binding to XOD. The docking results of 4APPY6O to XOD were then compared to those of uric acid and allopurinol results. Figure 2 showed the docking results of 4APPY6O to XOD compared to uric acid and allopurinol docking to XOD (7). A distinct difference in the hydrogen bonds of 4APPY6O binding to XOD occurred at the hydroxyl group to the e-amino group of Arg-880 and a hydrogen bond to the a-amino group of Thr-1010 (7). This of course is not surprising given the fact that there is an -OH group at position-2 of the 4APPY6O pyrimidine ring.



A)

B)

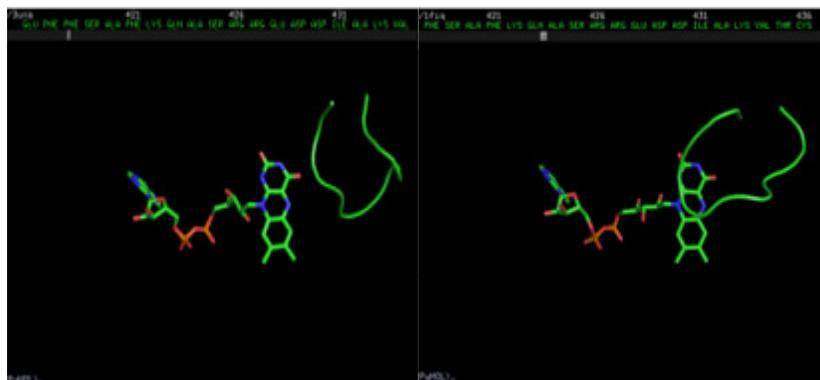


C)

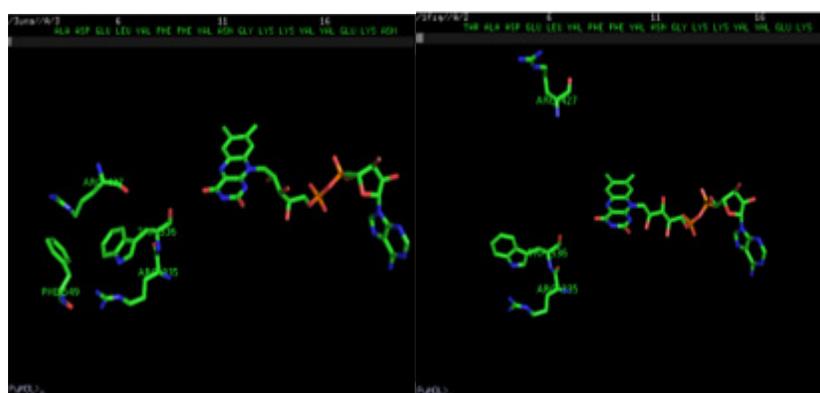
D)

Figure 2. The docking results which showed the hydrogen bonds and hydrophobic interaction results of 4APPY6O binding to XOD (D) compared to the hydrogen bonds and hydrophobic interactions that occurred for uric acid bound XOD crystal structure (A), the docking results of uric acid binding to XOD (B), and the docking results of allopurinol structure to XOD (C).

The XOD, also known as xanthine oxidoreductase, exists in two catalytically active forms, the oxidase form (XO) and the xanthine dehydrogenase form (XDH). Figure 3 demonstrates the transition of XDH form to the XO form.



(A). The residue loop of Gln-423 to Lys-433 in the XDH form on the left and the XO form on the right.



(B). The difference in the four-residue cluster can be seen above in the XDH form (left) and XO form (right).

Figure 3. Carbon atoms of the residues involved in the transition from XDH form to XO form.

When the XDH form is converted to the XO form, three major changes in its structure occurred. First, formation of a disulfide bond between Cys-535 and Cys-992 occurred; second, a major structural shift (movement) of a ten-residue loop (Gln-423 – Lys-433) at the NAD binding site occurred. In the XDH form the loop does not block the NAD binding site, but when it transitions to the XO form, the loop has moved to now block the NAD binding site, no longer allowing NAD into the active site in the XO form. The third major structural change observed was the disruption of a residue cluster containing Arg-335, Trp-336, Arg-427, and Phe-549 close to the FAD binding site. This cluster is no longer compact when it transitions into the XO form (8). The XOD enzyme which was purchased from Sigma Aldrich that was part of this work was the XO form.

Methodologies

The molecule, 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol (4APPY6O) and the enzyme Xanthine Oxidase (XOD) were purchased from Sigma Aldrich Chemical. For the computational study, the structure of the 4APPY6O was created from Spartan software by Eric Colwitz (7), and

the XOD structure was downloaded from PDB file of XOD complexed with uric acid (3AMZ) (2).

The purpose of this study was to evaluate if the compound, 4-amino-1H-pyrazolo[3,4-d] pyrimidine-6-ol (4APPY6O), will bind to XOD since its peripheral structure is somewhat similar to that of the allopurinol, a known drug that was used to prevent gout. If the 4APPY6O is able to bind to XOD, then in the following experiments the binding constant (K_d) would be measured. If a small molecule like 4APPY6 is able to bind XOD, then it is a potential XOD inhibitor or a drug for treating uricemia diseases.

To establish the binding of 4APPY6O (the ligand) to XOD, the Saturation Difference NMR Spectroscopy (STD- NMR) was used. In a NMR sample tube, the concentration of the ligand was made 10 mM 4APPY6O (0.760 mg) and that of the protein was 0.10 mM monomers (14.15 mg) in 20 mM Phosphate buffer, 5 mM NaCl, pH 5, 100% D₂O. The ratio of ligand (L) to protein (P) is 100:1 (5, 6). The ratio of 100:1 (ligand:protein) represents an excess of $[L]_t \gg [P]$, or a saturation point where the ligand signal is maximal and cannot get any bigger. In the STD-NMR experiment, at the ratio 100:1 (L:P ratio), the mixture of ligand and protein NMR was recorded at off-resonance signal ($I_{\text{off-resonance}}$), where the soft irradiation was at +40 ppm, essentially the NMR peaks, either from the ligand or protein, at this region are the same as the NMR peaks of the regular NMR spectrum. The soft irradiation of the on-resonance ($I_{\text{on-resonance}}$) NMR signal was set at -2 ppm, where methyl groups from the protein reside but no NMR peak of the 4APPY6O exists. The ¹H STD-NMR signal is obtained by taking the difference of the two NMR signals ($I_{\text{STD}} = I_{\text{off-resonance}} - I_{\text{on-resonance}}$). In the $I_{\text{off-resonance}}$ signal, the NMR spectrum is identical to the normal NMR spectra of the ligand plus the protein, though, the protein signal is essentially missing because its concentration is 100 times less than that of the ligand ($[L]_t \gg [P]$). In the $I_{\text{on-resonance}}$ NMR signal, NMR peaks of the ligand that do not bind to XOD will be exactly the same size as the NMR peaks of the $I_{\text{off-resonance}}$, thus they will be subtracted to zero. Whereas, NMR peaks of the ligand that do bind to the XOD will be of smaller intensity and they will subtract to non-zero value. The protein background NMR peaks of course is shown to be more pronounced in this case after subtraction. Figure 4 will demonstrate how the I_{STD} is obtained. Also, Fig. 4 will demonstrate how a protein is bound to the ligand in the fast exchange domain, how the spectrum at each domain is obtained due to the saturation effect, and finally how the I_{STD} is obtained.

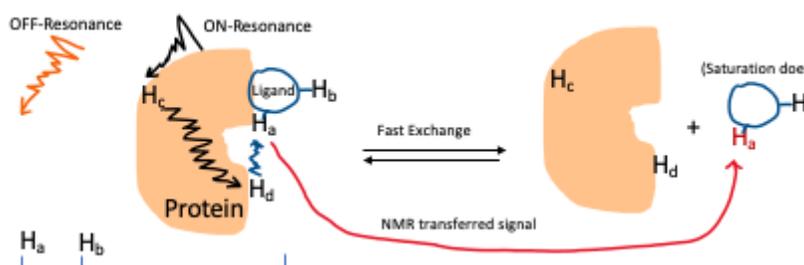


Figure 4. Demonstration of Ligand binding to Protein in the fast exchange domain. This figure (Fig. 4) demonstrates how the protons on the protein H_c is saturated, how the saturation effect occurs at H_d , how the saturation is transferred to H_a of the free ligand. Finally, how the STD-NMR signal is obtained.

To measure the binding constant K_d of the 4APPY6O to XOD, the STD-NMR experiments would be run exactly like the ones for the ratio of 100:1 (L:P), except that the ratio will be varied. For example, the ratio of L:P would be varied over the range as 5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1, 100:1, and so on. The XOD concentration would be kept constant at each point at 0.10 mM, and the ligand concentration would be varied according to the ratio given, keeping the buffer and pH to be the same. From these experiments, the K_d of 4APPY6O to XOD would enable it to be calculated (9).

The second part of the NMR experiments were to measure the ^1H STD-NMR peak intensity of the peak at 8.02 ppm according to the ratios given. At each L:P ratio (e.g., 5:1, 10:1, 15:1, etc) the ^1H STD-NMR peak percent would be measured. The ^1H STD-NMR peak would be measure the same way as the 100:1 (L:P) ratio, holding the XOD concentration constant at 0.10 mM, while the ligand concentration will be changed depending on the given ratio. In these STD-NMR experiment, buffer, salt and pH were to be maintained the same way as in the L:P ratio of 100:1. Because at lower L:P ratio the percent STD-NMR peak will be lower, and successively the percent STD peak will get bigger and bigger as the ratio goes up higher (9). These STD-NMR experiment will be repeated for each ratio given at the methodology until the ^1H STD-NMR peak reaches saturation or the same level to the L:P ratio of 100:1.

To measure the percent STD-NMR peak, for each ratio study, one typically calculates the amplification factor (A_{STD}) by divides the I_{STD} by the $I_{\text{off-resonance}}$ and multiply by the ratio of ligand to protein according to the equation below (9).

$$A_{\text{STD}} = \frac{I_{\text{STD}}}{I_{\text{Off-Resonance}}} \times \frac{[L]}{[P]}$$

When all the ratio (L:P) are studied, each ratio will yield one I_{STD} with different intensity because the amount of [L] is different, thus a different percentage of the A_{STD} will be obtained. When one plots the A_{STD} versus the ligand concentration [L] according to the equation below (Michaelis-Menten Equation), one will get a curve that is undersaturation at low [L] and becomes increasingly saturated at high [L], where the A_{max} is the maximum amplification factor. Thus, the K_d could be calculated by fitting directly the experimental data to the equation below (Michaelis-Menten Equatin) by using Microsoft Excel (9). Or one can linearize the equation as a Lineweaver-Burt plot and obtain the K_d at the slope when plotting $1/A_{\text{STD}}$ versus $1/[L]$, where the y-intercept equals $1/A_{\text{max}}$.

$$A_{\text{STD}} = \frac{A_{\text{max}}}{K_d} \frac{[L]}{1 + [L]} \quad \text{Michaelis-Menten Equation}$$

$$1/A_{\text{STD}} = (K_d/A_{\text{max}}) \cdot (1/[L]) + 1/A_{\text{max}} \quad \text{Lineweaver-Burt Equation}$$

Results

The chemical structures below showed the allopurinol structure (left) and that of the 4APPY6O (right), with the numbering system according to Truglio et al (10).



In the 4APPY6O structure (right) only the H₇ is NMR active, thus there is only one NMR signal at 8.02 ppm, corresponding to this H₇ shows up. That was what it was observed for this ligand.

The results at saturation at the ratio of 100:1 indicated that there is good binding between the 4APPY6O to XOD. There is only one NMR peak that was observable for the 4APPY6O at 8.02 ppm, when run alone without the XOD. This was the NMR peak corresponding to H₇ on the 4APPY6O. When the ¹H STD-NMR was run for 4APPY6O mixed with XOD at the ratio of 100:1 (L:P), a ¹H STD-NMR peak was observed at 8.02 ppm (Ioff-resoance, green trace). Figure 5 showed that when the off-resonance NMR spectrum (green trace) is subtracted from the on-resonance NMR spectrum (typically not shown) the ¹H STD-NMR spectrum is obtained (purple trace). The ¹H STD-NMR peak appeared at 8.02 ppm with reduced intensity and at the same frequency as the NMR peak of the green trace at 8.02 ppm. This indicated that indeed the ¹H STD-NMR peak arose from the H7 of the 4APPY6O. The first and second traces from the top of Fig. 5 served as negative checks for the STD-NMR spectrum. The blue trace was a regular ¹H NMR spectrum of 4APPY6O without protein, which indicated that the active NMR peak reside at 8.02 ppm. The red trace, a ¹H STD-NMR, indicated that in the absence of the enzyme XOD there was no binding, thus no ¹H STD-NMR peak showed up.

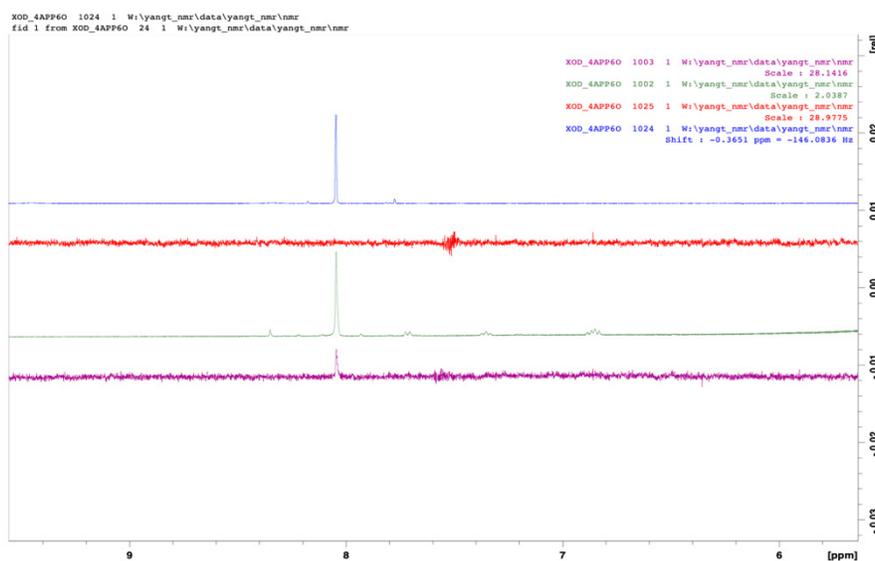


Figure 5. ^1H NMR spectrum of 4APPY6O. ^1H NMR spectrum of 4APPY6O without XOD, peak at 8.02 ppm (Blue). ^1H STD-NMR spectrum of 4APPY6O without XOD; no XOD present, no binding, so no STD peak (Red). Off-resonance ^1H STD-NMR spectrum of 4APPY6O with XOD, peak at 8.02 ppm (Green). ^1H STD-NMR spectrum of 4APPY6O with XOD present, binding occurred, so STD present at 8.02 ppm (Purple). The second ^1H STD-NMR spectrum from the top was used as a negative test check.

When the ^1H STD-NMR results from the second part was measure, in order not to see any precipitate of the ligand, the pH was adjusted too high (pH~12). At pH 12 the 4APPY6O and XOD mixture was clear. And when the pH was brought down to around 7 a precipitate was formed. No STD-NMR results were obtained at pH~12; more than likely the XOD was already denatured at high pH. A second sample was made and in order not to see the precipitate the pH was adjusted to 3.5. At the pH 3.5 still there was no STD result, again the XOD was already denatured that was why there was no STD results. A third sample was made and the pH was adjusted to 4.5-5 with no precipitate. At the third sample, there was ^1H STD-NMR peak observed. Figure 6 showed the ^1H STD-NMR of that results.

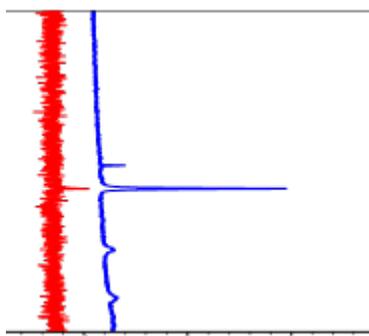


Figure 6: Figure 6 showed the ^1H STD-NMR resulted of the third NMR sample. This data showed that the ^1H STD-NMR came back with the correct pH range.

However, at this point the dry compressed air that feed to the NMR spectrometer had already broken, and the NMR experiments had to be watch closely in order not to get the spectrometer wet. Besides, the NMR spectrometer had to be run with lesser amount of time 3.5h or 512 scans for each ratio compared to the past with 7h or 1024 scans. Because the time or number of scans were uneven comparison of STD results to past experiments could not be made. Due to time constraint, the NMR experiments could not be continue. Nonetheless, the ^1H STD-NMR peak at 8.02 ppm was observed clearly at the saturation point.

Conclusions

The work done at the beginning of the project definitely showed that a ^1H NMR peak at 8.02 ppm had a STD peak at saturation. It showed that the 4APPY6O does bind to XOD, and that the STD NMR peak is significant large to get the A_{STD} without any problems. In the second part of the work, the A_{STD} could not be workout for the various L:P ratio planned because the pH was

not correctly adjusted. Besides, there was not enough time from the student stand point, and the NMR spectrometer was uncooperative.

Acknowledgements

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Investigating gene-environment interactions affecting methylmercury toxicity during embryonic development in zebrafish

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Abstract

Methylmercury (MeHg) is a common environmental contaminant in Wisconsin, and prenatal or early exposure to MeHg can cause developmental abnormalities. My research involves investigating gene-environment interactions of MeHg metabolism in embryonic development. MeHg is cleared from the body via oxidative stress metabolism pathways involving glutathione (GSH). The amount of time MeHg stays in the body varies across individuals. This diversity may be due to genetic polymorphisms in genes involved in MeHg metabolism. Certain alleles of GSH-related genes relate to elevated blood MeHg levels in adult humans; how these alleles affect MeHg metabolism in development is not well understood. This project aims to produce and validate mutant zebrafish lines with homozygous loss-of-function mutations in the *gclm* gene, a GSH-related gene associated with MeHg metabolism, using CRISPR-Cas9 reagents and high-resolution melt analysis genotyping (HRMA). The methods for this project were confirmed using CRISPR reagents targeting tyrosinase (*tyr*), a gene involved in pigment production. These results enable future experiments to characterize genetic influence on MeHg toxicity during development.

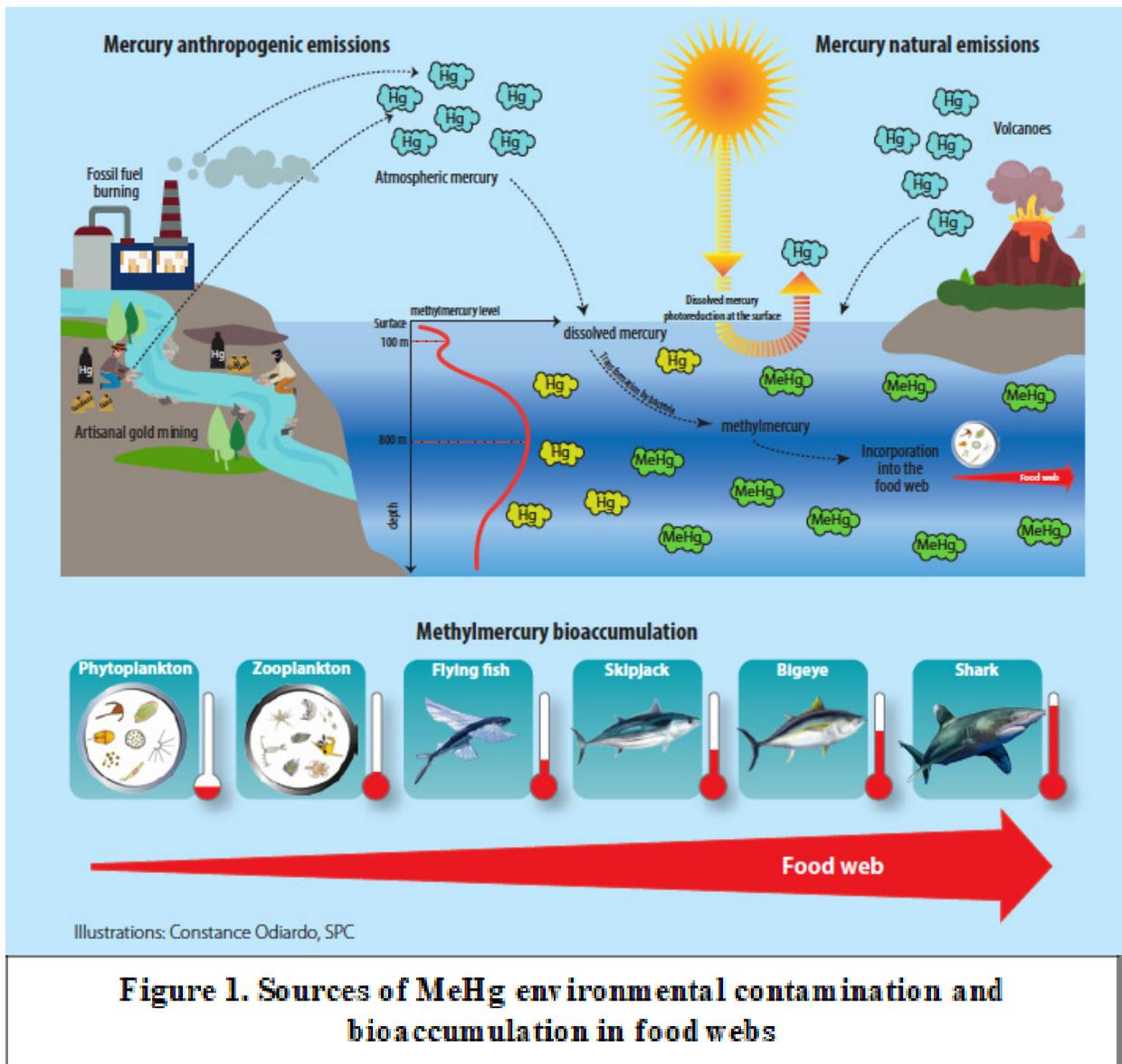
Introduction

Methylmercury; toxicology and metabolism

Methylmercury (MeHg) forms when bacteria react with inorganic mercury from industrial byproducts, water, air, soil, and plants. Bioaccumulation of MeHg can occur in aquatic food chains; small concentrations in small aquatic organisms accumulate to higher concentrations as the small organisms are eaten by larger fish. Once these fish are consumed, ingestion of the MeHg that has been accumulating throughout the food chain is released into the human body (Harding G, 2018). Once MeHg is in the body, it produces disulfides with a high chemical affinity to sulfhydryl groups from proteins. These disulfides are important for making protein structures and enzyme functions nonspecific as well as oxidative stress.

Atmospheric mercury comes from the burning of fossil fuels, mining, soil erosion, volcanic ash, and other environmental factors (Harding G, 2018) (Figure 1). Bacteria react with inorganic mercury in the water to form MeHg. When bacteria are digested by small organisms like phytoplankton, MeHg begins to accumulate in the food chain. As subsequent organisms digest smaller ones, the bioaccumulation of MeHg increases, leading to higher levels of MeHg in

aquatic organisms consumed by humans.



MeHg is a common environmental pollutant in the Great Lakes area and is a known toxin to prenatal health and development. The upper level of MeHg considered to be safe is 5.8 parts per billion (ppb) for newborns in a bloodspot taken at birth. In the Lake Superior region, 1% of umbilical cord blood spots have levels above 58 ppb and a maximum of 211 ppb (McCann, 2011). Prenatally exposed children can display a wide range of effects from subtle developmental delays commonly associated with autism spectrum disorders to severe cerebral palsy (Castoldi et al., 2001). The effects of low-dose MeHg exposure are subtle and can include impaired motor function and sensory defects (Grandjean et al., 1999; Grandjean et al., 1997). In the nervous system, MeHg has been shown to inhibit protein synthesis, disrupt microtubule assembly, increase intracellular Ca^{2+} , interfere with neurotransmitter function, and induce oxidative stress (Sanfeliu et al., 2003). Understanding the developmental effects of MeHg toxicity can inform

best prenatal health practices for pregnant mothers and their babies.

MeHg is removed from the body as a glutathione (GSH) conjugate. When MeHg enters a cell, it will bind to GSH, and the complex becomes a substrate for proteins that mediate cellular export of GSH conjugates out of the body (Ballatori, 2002). The amount of time MeHg remains in the body varies greatly across individuals from 30 to 150 days (Caito et al., 2018). This variation is due in part to genetic polymorphisms in the genes associated with the regulation of cellular MeHg. While the effects of prenatal MeHg exposure are well characterized after birth, this project aims to focus on the effects of MeHg exposure during prenatal development. These results will allow us to further understand gene-environment interactions of MeHg during prenatal development.

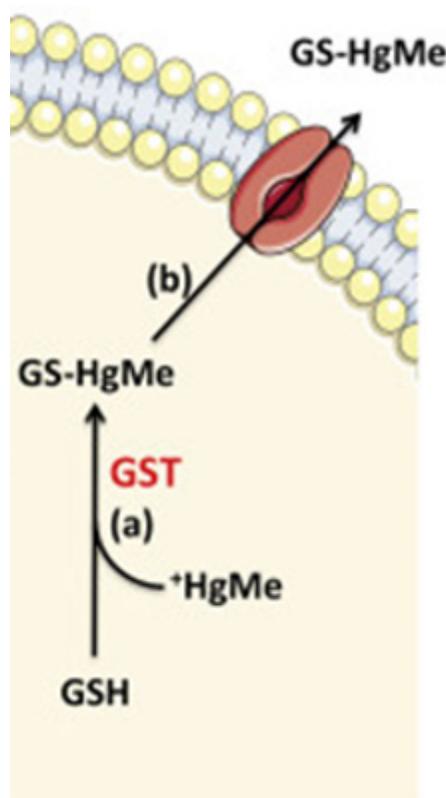


Figure 2. Pathway involved in the transportation of MeHg out of a cell encoded by the *gclm* gene. MeHg (depicted as HgMe) binds to a GSH conjugate to create a GS-MeHg complex (depicted as GS-HgMe) and is then transported out of the cell through the use of a regulatory membrane transport protein. *GCLM* is involved in production of GSH. Adapted from (Farina and Aschner, 2019).

GCLM is a gene that encodes a glutamate-cysteine ligase regulatory subunit involved in the synthesis of glutathione (GSH) from L-cysteine and L-glutamate. To remove MeHg from a cell, MeHg is attached by an enzyme to GSH and then transported as a GS-MeHg complex out of the cell (Carvan et al., 2004) (Figure 2). *GCLM* is involved in regulating GSH production and thus

regulating the cellular capacity to remove MeHg from the cell. Variants in the *GCLM* gene are found in the human population, leading to differences in the amount of time MeHg remains in the body.

Zebrafish as a Model Organism

We use zebrafish as our model organism due to its wide range of technical advantages in research. Zebrafish have a similar brain development and similar genomic DNA to humans. Zebrafish can be used for gene editing with CRISPR reagents (Carvan et al., 2004), an important feature for testing gene-environment interactions. Zebrafish have a transparent developmental stage that allows for characterization of visual mutations and aids in the process of fin-clipping for DNA isolation. Zebrafish also have a rapid early development and develop into mature adults 3 months post fertilization. Zebrafish yield a large number of embryos, enabling large sample sizes for experiments (Carvan et al., 2004). They are commonly used in biological research due to their small size, being easy to work with, and relatively inexpensive organisms. Of note for these experiments, zebrafish have a single homolog of *GCLM* (*gclm*).

Zebrafish also have characterized genetic mutations that produce visible phenotypes. For example, tyrosinase (*tyr*) is a gene involved in the pigment production pathway (Figure 3). Blocking the function of this gene inhibits the pathway to produce melanin pigment, which results in little to no pigment production. Mutation in this gene is characterized as a visible phenotype (i.e. unpigmented embryos) that is used to confirm our microinjection techniques and reagents before targeting the *gclm* gene, which likely results in a non-visible phenotype. Brightfield microscopy can be used to visualize the loss of pigment production as a result of *tyr* mutation from Cas9-gRNA microinjection.

Tyrosine Catabolism

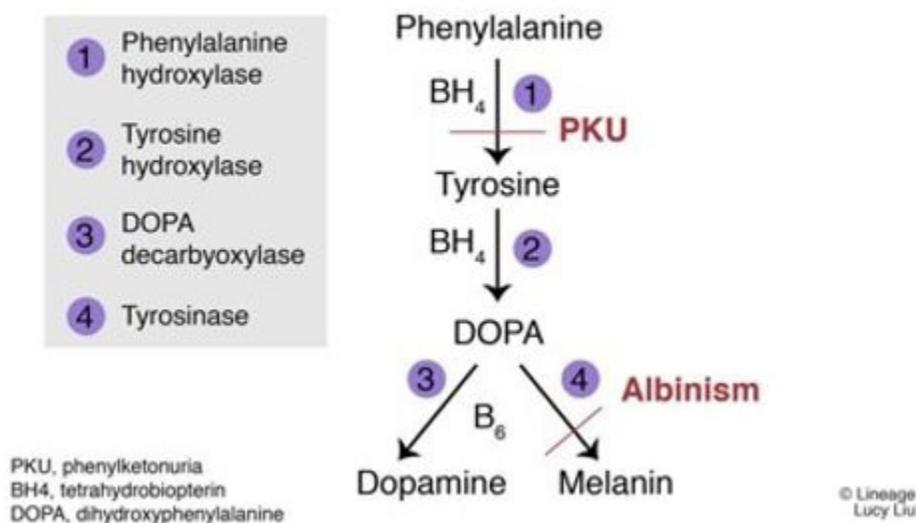


Figure 3. Pathway involved in the production of melanin. At step (4) in the pathway, tyrosinase acts to produce melanin. Blocking tyrosinase function results in albinism or an absence of melanin. Adapted from (Liu et al., 2021).

Methods

Breeding and Embryo Collection

Zebrafish of the EK strain were used for these experiments. Adult males and females are set up in breeding tanks the night before anticipated embryo collection to allow the zebrafish to acclimate in the tanks. A divider is inserted into the breeding tank to ensure timed breeding occurs. Timed breeding is important for this project to ensure that reagents are being injected into the single-celled embryonic stage. The divider is removed in the morning after the transition from the dark to light cycle. This transition is crucial for breeding since zebrafish breed shortly after the transition to the light cycle. Embryos are collected 10 minutes after the divider is removed. The embryos are rinsed to remove any debris and then placed into a petri dish with E3 solution prior to microinjection.

CRISPR/Cas9

CRISPR/Cas9 technology was developed from endonucleases found in bacterial and archaeal genomes. This technology involves sequence-guided DNA cutting by the Cas9 enzyme (Li et al., 2016). CHOPCHOP web tool was used to design a gRNA sequence to match the target DNA sequence in the gene of interest (Labun, 2019). gRNA was synthesized using a NEB EnGen kit (BioLabs). Using the synthesized gRNA with CRISPR/Cas9 reagents results in a mutation (insertion or deletion) that can lead to a loss-of-function within the gene (e.g. frameshift leading to premature stop codon).

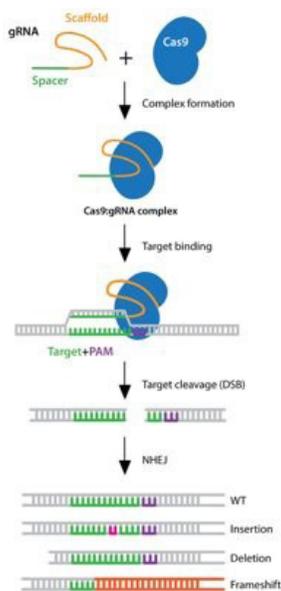


Figure 4. Diagram of CRISPR/Cas9 gene editing. Cas9 protein binds with the scaffold on targeted gRNA, forming a Cas9/gRNA complex. The spacer in the complex then binds to the target sequence adjacent to PAM sequence. Here, a double-stranded break is created, and the target sequence is cleaved resulting in an insertion, deletion, or frameshift mutation. Adapted from CRISPR Guide at Addgene (<https://www.addgene.org/guides/crispr/#web-references>).

Microinjection

The microinjection process involves combining CRISPR/Cas9 protein with gRNA, designed to match our target DNA sequence, nuclease-free water, and phenol red dye. Phenol red dye enables visualization of the injection of reagents into the embryo. Once the reagents are combined, the contents are pipetted into a capillary needle and inserted into the microinjection apparatus. When the needle was secure, a specialized slide was placed on the microscope and a drop of mineral oil was added to the center of the circle. The needle was cut using forceps to obtain a diameter of injected reagents of 1nL. Embryos were added to the agarose well-plate with E3 solution and were injected with the CRISPR/Cas9 and gRNA reagents. After the injection of CRISPR/Cas9 and gRNA reagents, embryos were transferred into a new petri dish with E3 solution. After completion of all injections, petri dishes were placed into an incubator set at ~28.5 degrees Celsius.

Fin Clipping

At 3 days post fertilization (dpf), zebrafish that were injected with *gclm* gRNA and CRISPR/Cas9 reagents undergo fin-clipping. 3-4 zebrafish were placed into a petri dish filled with E3 and 2-4 drops of tricaine solution to anaesthetize them. Once the zebrafish were anaesthetized, a single fish is placed onto a petri dish lid covered with scotch tape and arranged under the microscope. A scalpel was used to cut the end of the tail off between the end of the tail and the return artery of blood flow. Forceps were used to collect the tail sample and placed into the corresponding PCR tube with NaOH and labeled with the fish number. Once finished, the scalpel and forceps were sterilized with ethanol and wiped dry with a Kimwipe. A short period of observation is necessary to make sure the artery was not cut, and the fish will not die from a loss of blood. The fish were collected and placed into a recovery dish filled with E3 solution. Once recovered, the fish were placed back into their corresponding well in the multi-well plate with E3 solution and placed into an incubator set at ~28.5 degrees Celsius (Wilkinson et al., 2013). The clipped fins are now ready for DNA isolation. PCR tubes were heated at 95 degrees Celsius for 10 minutes with a cool-down period at 4 degrees Celsius for 30 seconds in the thermocycler. 2 uL of 1M Tris pH 8.0 was added to each PCR tube to neutralize NaOH, then spun down in a centrifuge. The supernatant containing the DNA was pipetted off, placed into new PCR tubes, and stored at -20 degrees Celsius (Meeker et al., 2007).

High Resolution Melt Analysis

High Resolution Melt Analysis (HRMA) genotyping is a quantitative analysis of a melt curve after polymerase chain reaction (PCR) amplification. HRMA measures small changes in DNA sequence by measuring the fluorescence signals in each sample and is reported to detect as small as a single base change in a DNA sequence (Wilkinson et al., 2013). These changes in fluorescence indicate a genetic difference between the samples, measured in Relative Fluorescence Units (RFU). PCR primers for the genes of interest are designed using the CHOPCHOP web tool (Labun, 2019). Synthetic DNA templates were used to model variations in HRMA signals of different PCR product lengths. PCR templates containing 5bp deletion and 10bp deletion model the expected difference in RFU signal due to a mutation of these lengths at the target site. The full-length template models the signal of the sequence with no mutation, matching wild-type/reference RFU signal. Synthetic templates were ordered as DNA oligonucleotides from IDT.

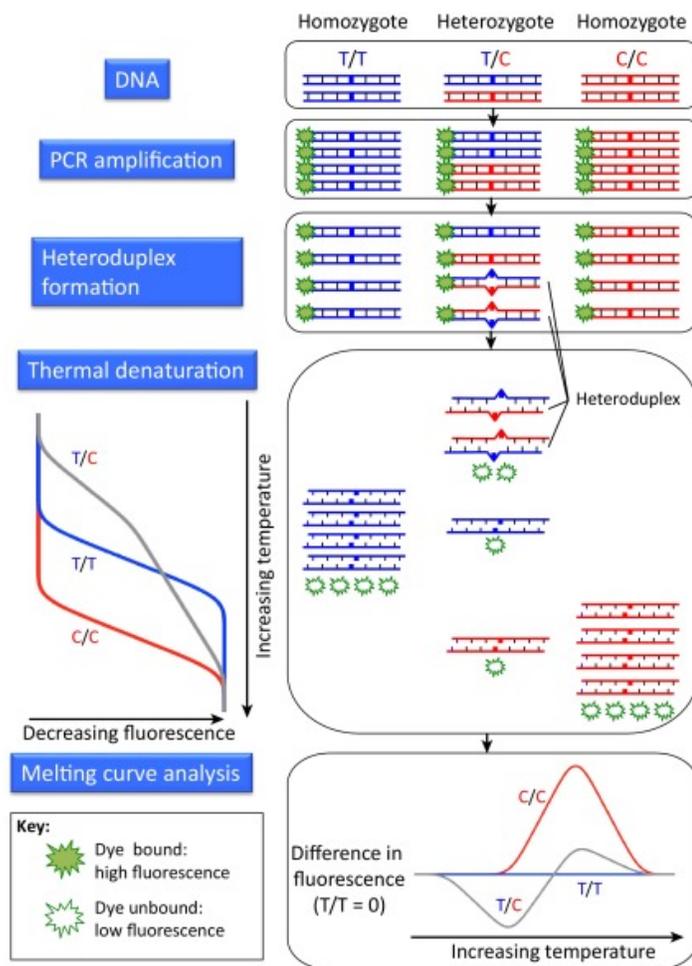


Figure 5. Diagram of HRMA procedure. PCR amplification makes copies of the DNA sequence. Then, the fluorescence dye is bound to the DNA and undergoes thermal denaturation, separating the two DNA strands. The fluorescence signals are measured against a specified reference sample to get the Difference RFU value. Differences in DNA sequence result in different denaturation kinetics, producing distinct patterns of fluorescence signal by genotype. Adapted from (Simko et al., 2016).

Results

CRISPR and HRMA genotyping were used to create knockout zebrafish lines. *tyr* mutants have a distinguishable difference in pigment production, most visible within the eye cells, making them more transparent and allowing individual eye cells to be more distinguishable (Figure 6). Reduced pigment production can also be seen along the spine and on the yolk. The results shown in figure 6 indicate mutation of the target gene, validating the microinjection technique and our process of developing CRISPR/Cas9 reagents.

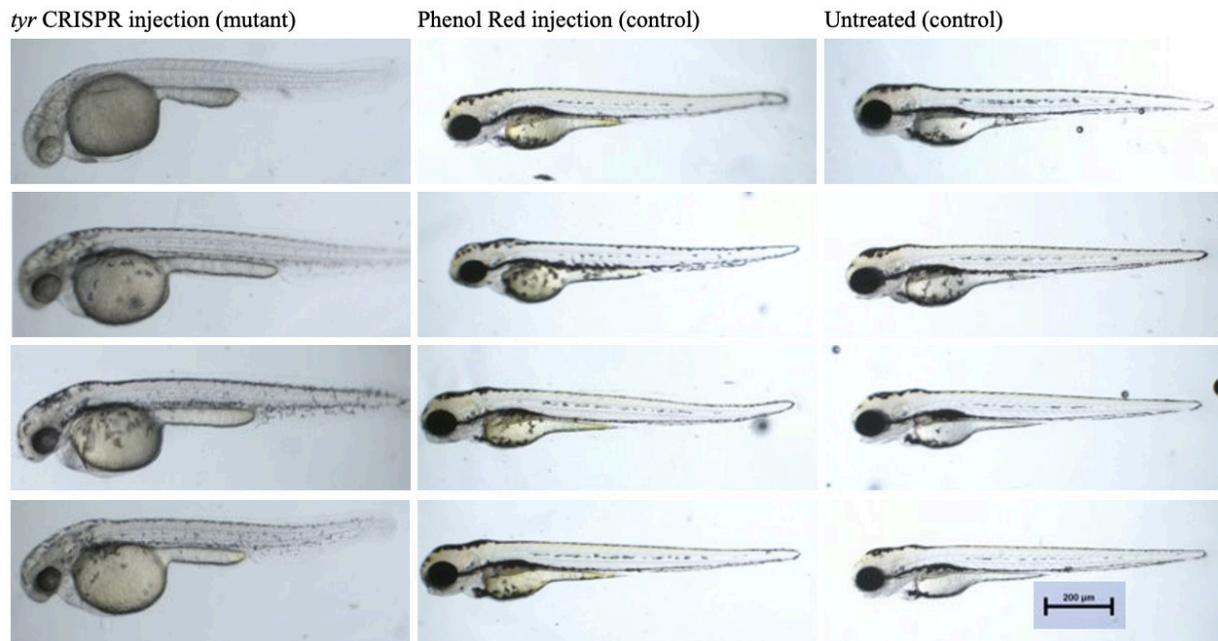


Figure 6. Zebrafish injected with *tyr* CRISPR reagents show pigmentation changes, indicating genetic mutation. The left column of zebrafish shows the *tyr* mutant phenotype; the middle column shows the phenol red phenotype (injection control); and the right column is wild-type (clutch control). The difference in pigment production is most distinguishable within eye cells. Images were taken with a Nikon SMZ1500 (C-DSD115) scope at 3 dpf.

Using HRMA genotyping, we were able to confirm *tyr* mutations from our CRISPR reagents. The *tyr* DNA sequences modeling 5bp deletion and 10bp deletion produced RFU differences of 0.06 compared with full-length wild-type DNA sequence (Figure 7). We thus predicted a similar CRISPR-induced mutation would have a similar difference in RFU. The HRMA genotyping experiment with *tyr* injected fish DNA observed a Difference RFU value of 0.065 (figure 8), suggesting a similar change in sequence to these altered DNA sequences.

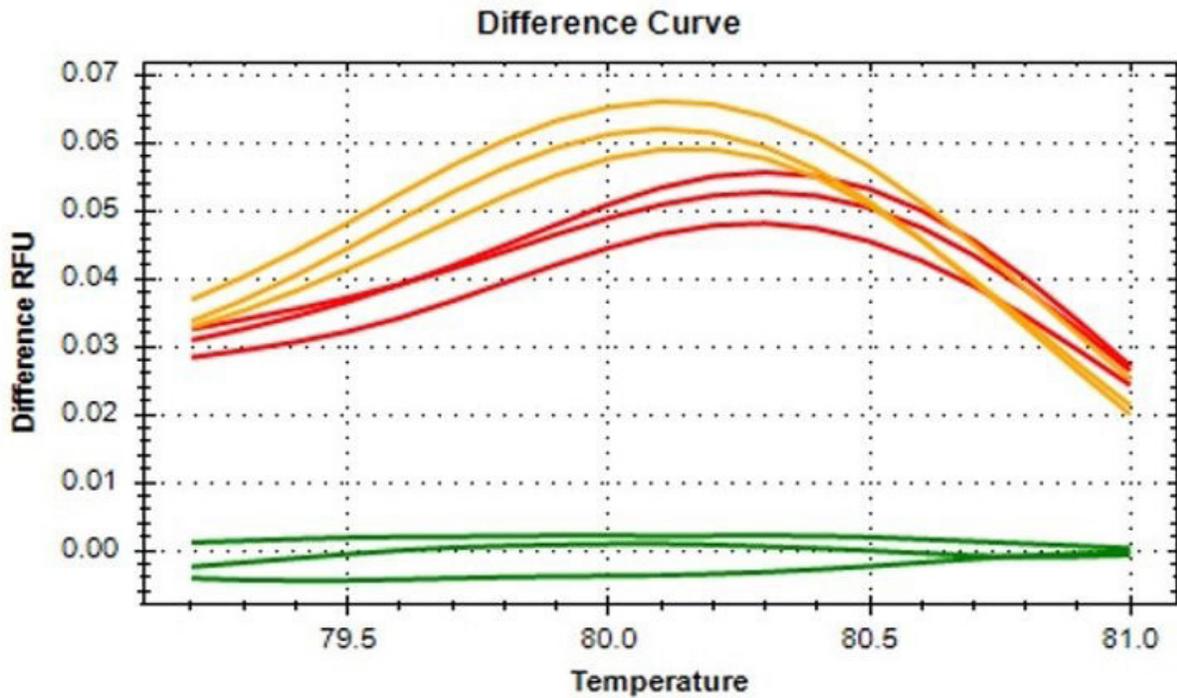


Figure 7. HRMA genotyping distinguishes between DNA templates for *tyr*. Primers for *tyr* were used with synthetic DNA templates with differing base pair lengths to model CRISPR-induced deletions (WT vs 5bp deletion vs 10bp deletion). Results show an expected Difference RFU value of 0.06 for CRISPR-induced deletions. (WT-green, 5bp deletion- yellow, 10bp deletion-red). Each line represents an individual HRMA reaction (technical triplicates). Values were referenced to the WT template.

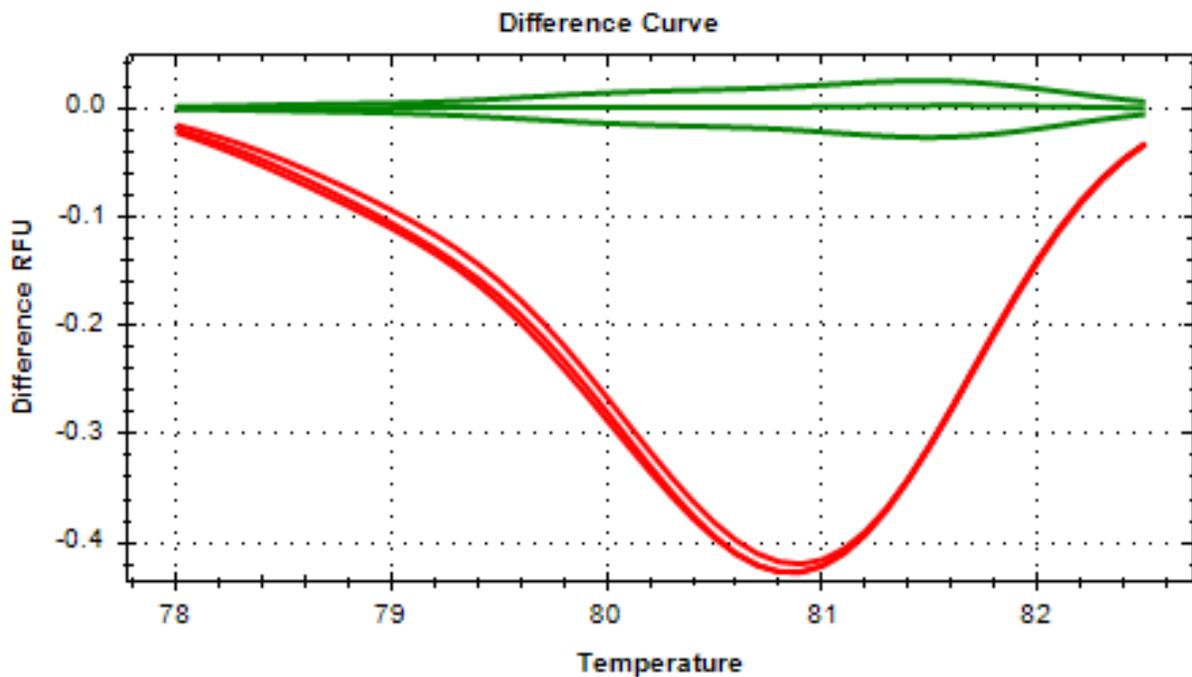


Figure 8. HRMA genotyping distinguishes between *tyr* mutants and WT fish. A portion of *tyr* mutants (red, n=24) had apparent distinguishable Difference RFU compared to WT (green, n=7) and phenol red control (blue, n=8). WT and phenol red control do not have a distinguishable Difference RFU. Each line represents DNA from an individual fish. Values were referenced to the WT control group.

Using HRMA genotyping, we were able to confirm *gclm* mutations from CRISPR reagents. The *gclm* DNA sequences modeling 10bp deletions produced RFU differences of 0.4 compared with full-length wild-type DNA sequence (Figure 9). We thus predicted a similar CRISPR-induced mutation would have a similar difference in RFU. The HRMA genotyping experiment with *gclm* injected fish DNA observed a Difference RFU value of 0.45 (figure 10A and 10B), suggesting a similar change in sequence to these altered DNA sequences.

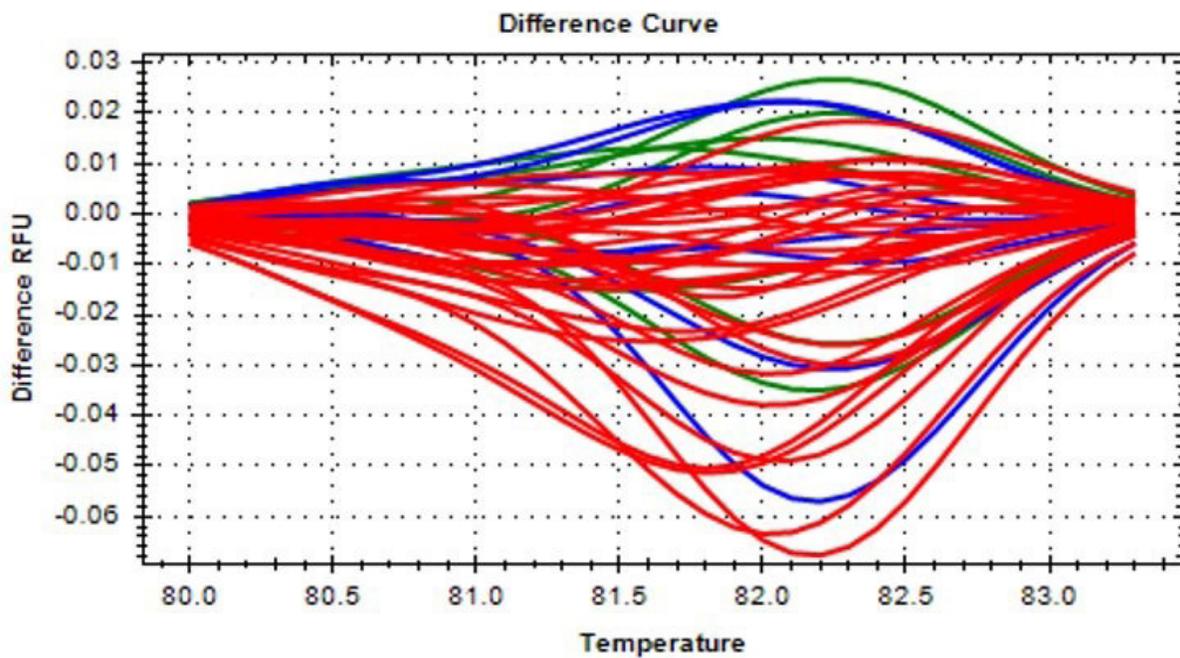


Figure 9. HRMA genotyping distinguishes between DNA templates for *GCLM*. Primers for *GCLM* were used with synthetic DNA templates to model CRISPR-induced deletions; WT (green), 10bp deletion (red). Results show an expected Difference RFU value of 0.4 for CRISPR-induced deletions. Each line represents an individual HRMA reaction (technical triplicates). Values were referenced to the WT template.

The first HRMA genotyping experiment with *GCLM* injected fish DNA observed a Difference RFU value of 0.4 (figure 10A), suggesting mutation similar to the 10bp template. The second HRMA genotyping experiment with *GCLM* injected fish DNA observed a Difference RFU value of 0.5 (figure 10B), suggesting a larger change in the sequence than initially suspected.

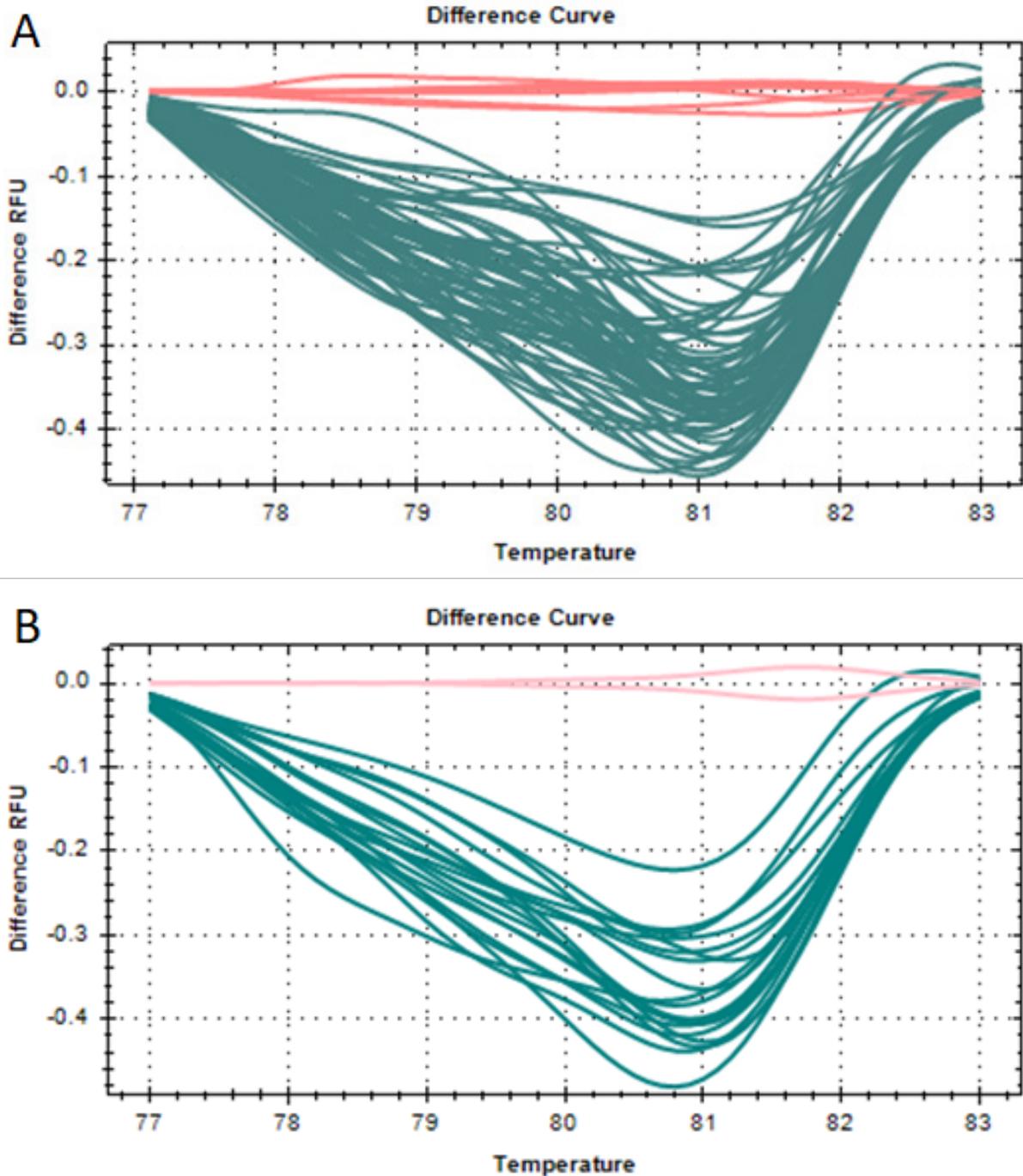


Figure 10. HRMA genotyping distinguishes between *gclm* mutants and WT fish. (a) A majority of *gclm* mutants (teal, n=62) had apparent distinguishable Difference RFU compared to WT (pink, n=8) with a value of 0.45. Values were referenced to the WT control group. **(b)** A portion of *gclm* mutants (teal, n=19) had apparent distinguishable Difference RFU compared to WT (pink, n=2) with a value of 0.45. Values were referenced to the WT control group.

Overall, the mutant samples are consistently different than the wild-type samples in both experiments, allowing us to be confident there is a mutation occurring. Validating these results

and identifying associated DNA sequence changes is the next step in creating a *gclm* knockout zebrafish line.

Discussion

The effects of MeHg exposure have been characterized after birth but are not well understood during human development. The overall goal of this project is to better understand neurological development in response to MeHg toxicity. We have been able to successfully create *gclm* mutations in zebrafish. We have raised the P0 generation that likely contain mutations of the *gclm* gene and are currently working toward characterizing mutation DNA sequences to identify loss-of-function alleles.

Future Directions

We intend to raise a transgenic mutant zebrafish line to test MeHg toxicity on homologous loss-of-function embryos and compare results to WT zebrafish. The next steps for this project are to create and validate a *GCLM* mutant zebrafish line by confirming the inherited mutation and determining the sequence of the mutation to ensure a loss-of-function allele. Outcrossing with WT zebrafish will be done to make F1 heterozygotes. The F1 generation will be raised and in-crossed to produce F2 homozygotes. After producing a transgenic mutant zebrafish line, we plan to test the effects of MeHg on social, motor, visual, and learning development of zebrafish compared to WT zebrafish.

A long-term future direction includes using human variants in *GCLM* on MeHg toxicity by combining our *gclm* knockout zebrafish with lines expressing human *GCLM* variants (collaboration with Carvan lab at UW-Milwaukee). Additionally, CRISPR/Cas 9 reagents and HRMA genotyping will be used to knock out additional genes related to GSH-production (*gstp1*, *gstp2*, *gclc*). This project will create a stronger understanding of the relationship between MeHg exposure and neurodevelopmental deficits.

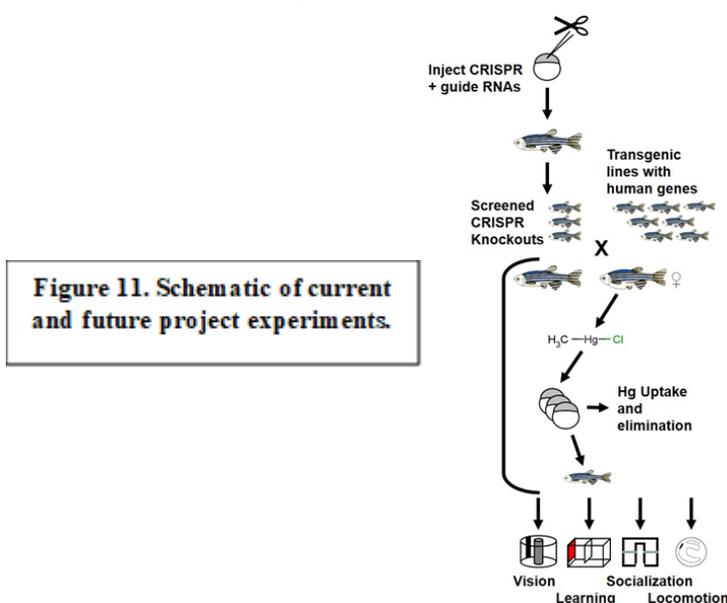


Figure 11. Schematic of current and future project experiments.

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Forged In Fire: Rehabilitation on The Fireline

A Mixed Method Analysis of Carceral Firefighting Programs

Cliff Hayes
Dr. Jason Spraitz

Abstract

This research seeks to achieve several goals, mainly to draw attention to a specific and understudied area of corrections—carceral firefighting programs. This study will achieve the above goal through a comparative analysis of carceral firefighting programs from the Western region of the United States, where such programs are most common. The analysis will focus on several key items, including, but not limited to: the number of program participants, level of training, and whether participants live in camps or stay in the prison. Additionally, the study will endeavor to highlight relevant legislation that impacts one’s ability to serve as a firefighter following their term of incarceration; one example is California’s recently passed Assembly Bill 2147 which allows for formerly incarcerated firefighters to use their training to serve in the same capacity upon release. Lastly, this study will highlight any transitional programs, should the state allow formerly incarcerated firefighters to be firefighters after their sentence is over as well as programs that have a framework for success in place, such as Arizona’s Phoenix Crew.

Introduction

Historic wildfires are becoming less historic, and more so a common annual occurrence. Images of burning landscapes, crimson sun, and skies to match are no longer confined to the West Coast, but now often find themselves in our media here in the Midwest. These images do not travel alone to our screens but are often accompanied by smoke from wildfires so massive it is difficult to imagine the scope of their destruction. These wildfires have stressed understaffed forestry and fire services, and with a mounting cost measured both monetarily, and in lives, states have turned to an unlikely population for help. This unlikely population was the focus of this project, and it is that person not often considered a hero often found amidst infernos such as those described above, the incarcerated firefighter. They are largely unrecognized and understudied in academia. Thus, the goal of this work is to build a base of knowledge for future studies, but more importantly highlight a gap in the study of criminal justice, and corrections. Specifically, the goal is to cover the following topics: what carceral firefighting programs are, how they rehabilitate, and the direction this topic of study can be taken in the future.

Where Carceral Firefighters Come From

This study’s geographical focus was the Western United States, as this region is both where the largest proportion of carceral firefighting programs are found, and is most often afflicted by wildfires. The following makes for a large, though incomplete, list of states with carceral firefighting programs:

State	Number of Personnel (Program Participants)
Arizona	240
California	3,700 (900 Fireline Qualified)
Colorado	160
Montana	15
Nevada	152
Oregon	345
Washington	300* (330 during the fire season 270 during the off-season)
Total:	
*Asterisks Denotes Approximation	

(California Department of Corrections and Rehabilitation [CADCR], n.d.; Colorado Correctional Industries [COCI], n.d.; Department of Forestry and Fire Management [DFFM], 2022; The Montana Department of Natural Resources & Conservation [MTDNRC], n.d.; Oregon Department of Forestry [ORDOF], n.d.; State of Nevada Department of Corrections [NVDOC], n.d.; Washington State Department of Corrections [WADOC], n.d.)

California has the largest carceral firefighting program out of all the states included in this study, and likely in the nation. With 3,700 members, 1,600 of whom reside in the state-run Conservation Camps, California has more program participants than the combined total of the six other states listed above. This specific population of incarcerated individuals makes up a relatively small portion of those involved in prison work programs, and an even smaller proportion of the total prison population. Despite the low population, this group can no longer be ignored or relegated to the sidelines as ecological disasters are becoming more common and we are increasingly relying on the labor of the incarcerated to preserve life and property. Further, these firefighting programs offer insight into different ways to approach rehabilitation and views of recidivism, while also challenging scholars and policymakers to review and refine the ethical considerations involved in prison work programs. The ethical considerations here are especially germane when considering the pay range for typical prison work programs, 12¢ - \$1.15 per hour, as compared to the firefighting programs which pay \$2.00 - \$5.00 a day with an additional \$1.45 - \$3.00 an hour when on fire duty (Prison Policy Initiative, 2017). One must also consider

that on top of the pay, there is the allure of getting to leave the prison to be in nature and in the community. There are few programs that can offer participants such pay and freedom, and these benefits are all at the cost of potential serious bodily injury or death.

Literature Review

Central to any prison work program is balancing the rehabilitative function and goals of the program, with the potentially exploitative nature of the program. Nowhere is this balancing act more noticeable or important than carceral firefighting programs, where death or life changing injury are an everyday risk while on job sites. What follows will be a synthesis of available literature on the rehabilitative nature of carceral firefighting programs, as well as their potentially exploitative nature.

Rehabilitation on the Fireline

An important feature of scholarship centered on carceral firefighting programs is the focus on the individual experiences of the inmates, camp staff, and administrative staff. This mode of study allows for insight into micro and macro-level thinking from the individual level all the way to the administrative and policy-making levels. An important step towards understanding the rehabilitative benefits of fire camps is that one must detach themselves from certain preconceived notions of what rehabilitation is. In Philip Goodman's work on the subject he challenges the reader to think not so much about the numbers (i.e., recidivism rates), but focus more on how the individual is experiencing rehabilitation and compare that to how the staff feels the individual is being rehabilitated (Goodman, 2012b). Largely, the view of both the camps' residents and of the staff is that the camp and the overall program are not focused so much on recidivism as an outcome, but rather a side effect. This is to say that the camp is an engine or enabler of change, and therefore makes the individual responsible for their own transformation. This responsabilization of the offender is seen as a focus on moral as opposed to actuarial reform (Goodman, 2012b).

The way in which an individual experiences rehabilitation varies from person to person, and even gender to gender. Among interviewees, a shared theme regarding the process of becoming physically fit emerged. The rehabilitative effects were found in both men and women, though more pronounced in women. The effects likely emerged from the idea that physical fitness and the changing body were a positive enabler of change and physically represented the transformation an individual goes through (Goodman, 2012b). The result is a disciplined hard worker who is both mentally and emotionally stronger as a result of the process of becoming physically fit (Goodman, 2012b).

Much of what is seen in the interviews conducted by Goodman might lead one to believe that responsabilization, the idea that one is the "... arbiter of their own fate..." is the main engine of rehabilitation. Other scholarship adds to the discussion and list of positive rehabilitative factors in fire camps (Goodman, 2012b, p. 450). Among the benefits found in the camps is an

older belief, that hard physical labor builds work ethic. Another benefit is the greater degree of freedom experienced by camp residents. One of the more interesting, and most important factors is found in the work of Lindsey Feldman. Feldman explores the topic of visibility in a carceral setting by focusing on Arizona's State Forestry Crews. Visibility, or being seen, outside the context of the carceral setting (i.e., by the community as opposed to corrections officers, other inmates, or other prison staff) is important. Visibility, for all intents and purposes is defined quite literally, though this definition fails to capture the most important facet and how it relates to furthering the understanding of carceral firefighting programs. Feldman describes prison labor as an "act of disappearance" where one not only physically disappears, behind the large walls and barbed wire fences of the prison, but also individually as one's former identity is replaced with a criminal one, if this has not already occurred (Feldman, 2019 p. 222).

The major rehabilitative factor of the fire camps then, is not simply that the firefighters are seen by the community, but rather how they are seen by the community and how this perceived image affects the individual's looking glass self (Feldman, 2019). Firefighters from different state programs described how it felt to be viewed positively by the community. Being described by some as "Heroes" can lead to conflicting feelings, as one must reconcile being viewed, and in some cases viewing themselves, as both a prisoner and a hero (Feldman, 2019; VanderPyl, 2021). Feldman further outlined two important facets which largely contribute to how one is viewed while in the community. The first is the clothing and equipment carceral firefighters wear and use, and the second is where they are viewed. In Arizona, the firefighters stay in a general prison unless they are out on a job as opposed to the conservation camps of California. The equipment and clothing that the Arizona State Forestry Crews use are non-descript and could be confused with any other Arizona firefighter's equipment or gear (Feldman, 2019). This is important because the lack of carceral symbology means that unless the community members are otherwise told, and in some cases in spite of what they are told when they are in the community working, carceral firefighters are simply viewed as who they are— firefighters, and not as what they have done.

In a recent article by VanderPyl (2021), a similar view is taken to rehabilitation in the camps. Though instead of visibility,—dignity and shame are examined. The ideas of dignity and shame as rehabilitative factors operate as both negatives and positives, just as visibility did in Feldman's work. VanderPyl asserts that one of the more insurmountable barriers to successful reentry has to do with the shame associated with a criminal record (VanderPyl, 2021). The experience of shame makes one begin to believe they are of little worth and impedes the ability for one to be rehabilitated (VanderPyl, 2021). Dignity has much the opposite effect. Increasing one's self-worth means social exclusion is less likely to occur. Allowing one to hold on to their self without replacing it with the criminal self-image improves one's ability to be rehabilitated (VanderPyl, 2021). When discussing the importance of visibility and dignity in one's personal experience of rehabilitation it must be pointed out that one of the most interesting features of the fire camps is the ever-present duality that exists. Incarcerated firefighters exist in a superposition of experiencing both shame and dignity, while being referred to as heroes and criminals simultaneously on job sites (Feldman, 2019; Goodman, 2012a; Goodman, 2012b).

Apart from the rehabilitative nature of the camps and the work itself, camp residents and firefighters staying in traditional walled prisons still have access to the rehabilitative programs one might find in a more conventional carceral settings such as substance abuse programs and reentry programs. Whether that type of programming should act as a model for rehabilitative incarceration going forward is unclear, and more research will need to be conducted before a more definitive answer can be given. Though the general consensus of prisoners and staff seems to be that while camps aid in rehabilitation, they are not without faults and most certainly are not free from claims of exploitation (Goodman, 2012a, Goodman, 2012b).

Exploitation: Service at a Cost

There is no lack of discussion when it comes to prison labor, whether it is warranted and useful, or if it is a step too far and exploitative. Few prison work programs are more appropriate for this discussion than carceral firefighting programs. Participants are regularly exposed to life threatening situations for mere dollars a day (Goodman, 2012a, Goodman, 2012b). A central feature of the argument about exploitation is the pay. Prison firefighters and regular firefighters both agree that the pay is inadequate compared to the work and the risk, while detractors argue that if the inmates were paid something close to minimum wage, then one might have the incentive to go to prison for the pay, meals, housing, and healthcare (Goodman, 2012a). Furthermore, a sentiment shared by staff and some prisoners, is that because they have committed a crime they are not deserving of greater pay (Goodman, 2012a). However, not all claims of exploitation relate to pay, with some focusing on the inmate-staff relationship. Inmates feel that many of their grievances were ignored or purposefully lost in the bureaucratic red tape. Not only this, but some felt that the better conditions and high demand for placement in the camps empowered staff to threaten camp residents with removal from the camp and placement back in the much less desirable walled prison, playing on the idea that there are countless others waiting to take your place if you don't want it (VanderPyl, 2021, Goodman, 2012a). Interestingly, as was seen when discussing rehabilitation, among inmates another duality emerges. Many incarcerated firefighters find their work to be both exploitative and useful at the same time, a theme seen across multiple states' programs (Feldman, 2019; Goodman, 2012a p. 361; VanderPyl, 2021).

Findings

The program characteristics compared in this study included the following: number of personnel; whether individuals were housed in the prison or in a camp; the level of training they received and or certification level if applicable; whether the clothing and equipment labeled the individual wearer or operator as an incarcerated individual; and lastly whether or not the state had a transitional program, or allowed the individual to seek employment as a firefighter post-release. These measures were chosen because they are central to the concept of visibility, shame, and dignity as described above. Additionally, it is important to know whether the state offers transitional programs or even allows former program members to be firefighters when

considering how meaningful employment impacts post-release outcomes, such as recidivism.

Housing: Prison or Camp

When looking at where firefighters were being housed we found that the majority were housed in the traditional prison and would leave from there to go out on jobs. The only three states that have dedicated camps are: California, Nevada, and Oregon. When considering the topic of where individuals are being housed, it is important to remember that if a state has dedicated camps, it does not necessarily mean that all program participants will be housed in such a facility. As seen above with California, less than half of all program members actually reside in the camps, while the rest stay in a traditional prison.

Training

When it comes to standards for training and certification levels, states may set their own requirements. However, many still defer to the National Wildfire Coordinating Group (NWCG), as the unofficial leader in Wildfire firefighting. Accordingly, when determining what level of training their incarcerated firefighters should receive, many states will require their firefighters meet the NWCG standard, or will have heavily structured their training around the NWCG training. Even still, no nationally recognized standard of training exists for incarcerated firefighters.

State	Level of Training or Certification
Arizona	NWCG Type I & II
California	FFT
Colorado	NWCG Red-Card
Montana	NWCG Type II
Nevada	NWCG FFBT S130-S190
Oregon	Receive training, not specified.
Washington	NWCG Type II

(CADCR, n.d.; COCI, n.d.; DFFM, 2022; MTDNRC, n.d.; ORDOF, n.d.; NVDOC, n.d.; WADOC, n.d.)

Training or Certification	Description
NWCG Type I "Squad Boss"	Leads small groups ≤ 7 and is responsible for their safety on wildland and prescribed fire incidents. Supervises resources at Type II level and reports to a Crew Boss or Specialty Boss.
NWCG Type II "Crew Member"	Performs fire suppression and fuels management duties in the most adverse climate, fuel, and terrain conditions. No supervisory responsibilities and is supervised by Type I.
NWCG Red-Card "Incident Qualification Card"	Interagency certification that is position dependent. Is issued upon completion of position specific training, and passing the positions required fitness test. Fitness tests vary depending upon if the position is: Arduous, Moderate, or Light.
FFT	Similar to Type I or II above depending on if an individual is an FFT-I or FFT-II. For the purpose of Conservation Camp participants must pass a physical fitness test to move into 1 week of classroom instruction followed by 1 week of field exercises.
NWCG FFBT S130-S190	S-190 is introduction to Wildland Fire Behavior and consists of 7 instructor-led training hours and 6-8 self-directed hours and must be completed prior to starting S-130. S-130 is the Firefighting Training Course and consists of 29 instructor led training hours with 8-12 hours of field exercises.

(National Wildfire Coordinating Group, 2021; California Governor's Office of Emergency Services: Fire Rescue et al., 2014)

Gear and Clothing: Labeled or Non-Descript

Regarding the gear these firefighters use, and the clothes they wear, only two states (California and Nevada) opt to have their firefighters clearly labeled as being incarcerated. The other states are more non-descript. This raises the question of how the rehabilitative experience varies from state to state, when considering how labeling affects the individual's experience in the program, and reception by community members.

Legislative Pathways to Employment & Transitional Programs

For many people participating in the myriad carceral firefighting programs, the end of their sentence typically means the end of their career as a firefighter. Why do those states that are both in need of firefighters, and who trained individuals in the very job they are in need of people for, might refuse to hire former program members. Further, firefighting provides meaningful employment whereby the individual goes to work knowing what they do makes a difference

in the lives of their fellow community members, especially when they save life or property. Accordingly, it can be argued that such employment acts as a protective factor against future criminal behavior. While three of the seven states have pathways to employment as firefighters, only two of the states in this study outline what these pathways are; California, and Arizona. In September 2020, California passed Assembly Bill 2147. This bill opens a pathway for former Conservation Camp program members to become firefighters outside of prison via the expungement of their criminal record. Much like the program itself, this expungement is only available for individuals who meet certain criteria. The Assembly Bill lays out this criteria in § II 1203.4b(a)(1):

- (A) Murder.
- (B) Kidnapping.
- (C) Rape as defined in paragraph (2) or (6) of subdivision (a) of Section 261 or paragraph (1) or (4) of subdivision (a) of Section 262.
- (D) Lewd acts on a child under 14 years of age, as defined in § 288.
- (E) Any felony punishable by death or imprisonment in the state prison for life.
- (F) Any sex offense requiring registration pursuant § 290.
- (G) Escape from a secure perimeter within the previous 10 years.
- (H) Arson.

In Arizona a similar step was taken in the form of the Phoenix Crew, a Type II Hand Crew consisting almost entirely of low risk offenders. This crew, founded in 2017, only consists of 20 members. However, it illustrates a concept that if brought to scale could offer an incredible opportunity at a “second chance” for many former program members. Moreover, the Phoenix Crew can set an example for other states to follow and expand upon.

Conclusion & Future Studies

The goal of the present study was largely to bring attention to an area of corrections which receives little of it. There are several directions that future studies may take this topic, and how they might affect change in this correctional practice. While it may, at the individual level, be more important to look at how the individual experienced rehabilitation regardless of post-release criminal activity, the truth of the matter is that in order to “sell” the idea of carceral firefighting programs, more traditional metrics will need to be explored. Thus the following relationships, or intersections, need to be explored: I.) What is the relationship between program participation and recidivism? II.) What is the relationship between program participation and employment? III.) What effect, if any, does program participation and employment have on recidivism? IV.) What is the outcome for those individuals who participated in the program, and successfully leveraged AB 2147, to gain employment as a firefighter post-release? Similarly, what of those who gained employment in the Phoenix Crew? V.) What effect does labeling play on the program participants’ overall rehabilitative experience, and does labeling create noticeably different effects post-release when compared to programs that do not label? VI.) Interstate and even intrastate transfers of prisoners are not uncommon, is this a practice that also takes place within carceral firefighting programs during the fire season? What about out of season?

The answers to these questions, taken with the findings from studies focusing on individual experiences, can help define carceral firefighting programs' place in the world of prison work and rehabilitative programs.

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Are Window Display Bans at Public Universities Constitutional?

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Background

Historically, public universities have been a means for its student population to achieve a sort of freedom that they had not yet experienced during their high school lives. Traditional college students are finally away from their guardians and have a chance to be around people their age for the duration of their degree. Being on a college campus allows you so many opportunities to express yourself in every facet of your life. That includes their ability to express themselves through their freedom of expression. In the United States, most campuses provide numerous channels for students to express their feelings on a wide variety of topics including politics, religion, and more. The United States Supreme Court has protected an individual's right to free speech not just in private residences, but also on public college campuses. The question I am posing is whether there is similar protection in college dorm rooms as there is in private residences. Specifically, are window display bans in student housing at public universities constitutional? There are a series of issues that must be addressed when talking about this question such as content neutrality, the differentiation between public and private forums, and captive audiences which will be addressed below.

Examples of Relevant University Policies

Ohio State: *“Windows must remain clear from obstruction and university window coverings need to be visible from the outside. Posting, hanging or otherwise displaying signage, lighting or other materials in or around the residence hall windows or on university window coverings is not permitted.”*¹

UW-La Crosse: *“Due to fire safety considerations and the possibility of damage to residence hall room windows, residents are prohibited from posting or hanging material on either side of residence hall windows. This includes but is not limited to signs, posters, flags, banners, paint, lights, post-its, or anything that partially obstructs windows.”*²

UW-Green Bay: *“Items posted on the outside of room/apartment doors or in room/apartment windows which are intended to demean an individual and create a hostile, and/or intimidating environment are prohibited.”*³

UW-Parkside: *“If an item is posted on or is visible in a student room door or room window*

that targets a specific individual or group in a harmful, harassing, or intimidating manner, the resident/s will be asked to remove it. If an item that is posted is generally discriminatory or distasteful, a discussion between the student and the Hall Director will occur.”⁴

UW-Platteville: *“no items may be adhered to or displayed in windows. The only items that may be in a window are twinkle lights around the perimeter of the window.”⁵*

UW-River Falls: *Residents posting items in a public manner that target specific individuals in a harmful, harassing, or intimidating manner or that are considered as hate/bias by UWRF will be required to remove these items.⁶*

UW-Eau Claire: *“To prevent damage to our facilities and maintain a welcoming atmosphere in the Residence Halls, residents may not display anything outside of their room, on a balcony, or in a residence hall window”⁷*

History of Free Speech

Since the United States constitution was ratified, there has been the notion of free speech. The United States' Constitution in the First Amendment states, “Congress shall make no law... prohibiting the free exercise thereof; or abridging the freedom of speech or of the press...”⁸ The United States' government has shared the view that free speech is an important right that United States citizens possess. This means that at the federal level, the government could not make any law abridging an individual's freedom of speech, religion, etc.

The first case that involved the bill of rights being incorporated at the state level was the case *Barron v. Baltimore* (1833). This case does not pertain to free speech particularly, but it involves a state question that determines if individuals have rights from the Bill of Rights that protect them from state governments. At this point, it was already clear that United States citizens had rights granted by the federal government, but it had not yet been established if those are the same with state governments. In this case John Barron claimed that the city of Baltimore, in doing construction, diverted the water flow in the harbor area where his wharf was. He sued the state for a portion of his financial damages. The trial court determined he was entitled to \$4500. The appellate court disagreed and struck that down. At the Supreme Court, they determined unanimously that the framers' intent in the bill of rights was an exclusive check on the federal government, not the state and federal jointly. Chief Justice Marshall thus came to the decision that the Supreme Court had no jurisdiction to hear this case. Marshall cited evidence from the defendants and Constitution and said, “This court, therefore, has no jurisdiction of the cause, and it is dismissed.”⁹ The major takeaway from this case was that, at the state level, the Bill of Rights was not incorporated, so the state government could infringe upon individual's rights. This would stand for continue for almost a hundred years.

Several years after *Barron v. Baltimore* the United States adopted the Fourteenth Amendment. This amendment became a vital piece of the United States Constitution as it relates to free speech and the Bill of Rights. The Due Process Clause of the Fourteenth Amendment says, “Nor shall any State deprive any person of life, liberty, or property, without due process of law”¹⁰. This clause in the Constitution is very similar to a part of the Fifth Amendment which was used for the *Barron* case. In fact, it is almost the same text. The Fourteenth Amendment explicitly mentions the state. While this could be used to describe a country, it can also be used to describe

literal states in the United States. This would become very important in *Gitlow v. New York* (1925) when the Court heard a case involving the incorporation of the Bill of Rights at a state level.

As with almost every right listed in the bill of rights, the right needs to be incorporated at the state level for it to apply to the states. Fast-forward 134 years to 1925 and a case can be found in the United States Supreme Court that would incorporate the First Amendment's free speech clause. In *Gitlow v. New York* (1925), the United States' Supreme Court made a ruling for the incorporation of the amendment. In *Gitlow*, Gitlow who was a reported socialist and was arrested for printing and distributing copies of the book the "Left Wing Manifesto". In that book there are calls to action such as strikes. According to New York Courts, this was a violation of the New York Criminal Anarchy Law. This law punished those who were in favor of a forcible overthrow of the government. Gitlow made the argument that no negative action followed his distribution of the manifesto. While working through the New York state court system he was convicted by the trial court, then affirmed by both the appellate and Superior Court of New York. This posed the question of if a state can punish political speech that advocated for the overthrowing of the government. The Court upheld the constitutionality of the state's right to punish those who threaten to overthrow the government, but there is a caveat to it. The United States Supreme Court requested that there should be a resulting action that negatively impacts the government. Justice Sanford said, "The sole contention here is, essentially, that as there was no evidence of any concrete result flowing from the publication of the Manifesto or of circumstances showing the likelihood of such result, the statute as construed and applied by the trial court penalizes the mere utterance, as such, of "doctrine" having no quality of incitement..."¹¹ The fact that the state could not provide evidence of unlawful action following the publication made this a constitutional issue caused by the state. Justice Sanford went on to say, "We may and do assume that freedom of speech and of the press which are protected by the First Amendment from abridgment by Congress are among the fundamental personal rights and "liberties" protected by the due process clause of the Fourteenth Amendment from impairment by the States."¹² By the conclusion of this case, the United States Supreme Court decided that the Fourteenth Amendment incorporated the First Amendment to be applied to the individual states.

Limitations of Free Speech

The liberties granted to people in the Free Speech Clause are not absolute. There are a large array of issues that came up and continue to come up in the Courts that created exceptions to individuals having a right to free speech. For instance, a case that exhibited this exact exception to free speech was *Schenck v. United States* (1919). Charles Schenck and an acquaintance distributed a pamphlet that alleged that the draft was a violation of the Thirteenth Amendment of the United States Constitution. The product they handed out encouraged people to avoid the draft for World War I. They allegedly encouraged that this be a peaceful protest of the draft. The issue with this is, according to the Courts, it is a violation of the Espionage Act of 1917. The pair sued the government citing that this is a clear violation of the First Amendment. The Supreme Court, in a unanimous decision written by Oliver Wendell Holmes said that this did not violate the First Amendment. The Court concluded that the publication could interfere significantly enough to undermine the efforts of Congress to protect the country through the draft. Holmes in his decision said, "The most stringent protection of free speech would not protect a man in falsely shouting fire in a theatre and causing a panic. It does not even protect a man from an

injunction against uttering words that may have all the effect of force.”¹³ In that moment, the Court determined that a couple people handing pamphlets encouraging people to boycott the draft would undermine Congress’s ability to perform the draft. Later, the United States’ Supreme Court determined additional ways to limit free speech through their court decisions. The mentioning of the case is strictly because of its relevance to free speech and expression and to provide an account of other methods previously used since it was overruled in the next case.

The extent to which the Court could limit free speech was then put to the test again with *Brandenburg v. Ohio* (1969). In this case, Brandenburg, who was the leader of the Ku Klux Klan in that Ohio area, was challenging a law that limited his speech. He was holding a Klan meeting in Ohio where he spouted racist rhetoric that could have turned dangerous had the members acted on what was said. Brandenburg was arrested and convicted under Ohio’s Criminal Syndicalism law. In essence, this law made it illegal to advocate for violence or unlawful methods of terrorism if it is to accomplish a type of political reform. The law also outlawed gatherings of any group whose purpose was to advocate for criminal syndicalism. Brandenburg challenged this law and his conviction on grounds that it was a violation of his First and Fourteenth Amendment rights. The Supreme Court determined that the Ohio law did in fact violate his right to free speech. To reason this they used a two-part test. The test as a whole reads, “Free speech and free press do not permit a State to forbid or proscribe advocacy of the use of force or of law violation except where such advocacy is directed to inciting or producing imminent lawless action and is likely to incite or produce such action.”¹⁴ First, there needs to be speech that occurs. Next, to meet this test, the speech needs to be proven to have incited violence. Looking at *Brandenburg*, the Courts partially overruled *Schenck* but not entirely. Since both methods are still used for interpreting free speech, the records do not directly say *Schenck* was overruled.

A couple years later a new challenge revolving around free speech made it to the Supreme Court. A young man, Paul Cohen, wore a jacket that read “FUCK THE DRAFT. STOP THE WAR”. Cohen opposed the war in Vietnam and used his jacket as a form of protest. His jacket allegedly violated California Penal Code 415 which outlawed an individual disturbing peace or quiet of any neighborhood or person... by... offensive conduct”.¹⁵ In a decision written by Justice John Harlan, the Court held that the California statute violated Cohen’s right to free speech and expression. In his decision he mentioned, “it is nevertheless often true that one man’s vulgarity is another’s lyric.”¹⁶ This is an important precedent that the Supreme Court of the United States set. This quote by justice Harlan is vital for the foundation of free speech in the United States.

School Speech

The previous cases were examples of speech that could be limited regardless of location. The following case involve student speech in a school setting. One of the first cases involving student speech was *Tinker v. Des Moines* (1969). Students from a school in Iowa planned a form of passive protest displaying their support of a truce in the Vietnam War. To do this, the students wore armbands with anti-war messages. The school caught wind of this and created a policy banning the wearing of armbands. If they did not comply with the request to remove the band, they would be sent home and suspended. A couple of students tested this and were suspended. Their parents sued the school for allegedly violating the First Amendment rights of the students

at the Des Moines Independent Community School District. In the district court, the court held that the school's actions were reasonable and dismissed the case. The parents appealed this decision to the United States Court of Appeals and affirmed the district court's opinion. The Supreme Court of the United States then weighed in on the case and determined that the prohibition against armbands in a public school did violate student's rights of free speech. In the opinion of the Court, written by Justice Fortas, students do not shed their First Amendment rights upon stepping onto the school property. Justice Fortas in the opinion wrote that schools can limit speech if it would, "materially and substantially interferes with the requirements of appropriate discipline in the operation of the school."¹⁷

Tinker set a solid precedent for schools. *Healy v. James (1972)* applied it to a college campus. A local chapter of a left-wing student group, the Students for a Democratic society, was the topic of discussion. Central Connecticut State College denied official student organization status to their local chapter of the Students for a Democratic Society. According to the school, it was denied because chapters of the organization were violent at other universities. This case eventually got to the Supreme Court of the United States and the Court held that the college could not deny the organization official status because they disagree with the ideas pushed by the organization. As it relates to free speech and college campuses, another important phrase was said during the *Healy* case. Justice Powell mentioned in his decision, "At the outset, we note that state colleges and universities are not enclave immune from the sweep of the First Amendment."¹⁸

After *Healy*, another prominent school speech case arose, *Bethel School District No. 403 v. Fraser (1986)*. This is a case where a high school student was running for a student elective office. While doing so, a friend of the candidate giving a speech used what some described as a graphic sexual euphemism. The school district found that it was a violation of their code of conduct and reprimanded the student on grounds that it violated their "substantially interferes with the educational process... including the use of obscene, profane language or gestures." As a result of this speech, Fraser was suspended from school. The United States Supreme Court held that the school could discipline a student for a lewd speech because it wasn't consistent with "fundamental values of public-school education."¹⁹ The wording of this quote would make it seem as though this could cover all public school, potentially even college level public universities. However, most speech that occurs in a residence hall doesn't have a forum similar to this case, nor would the student be reprimanded if they did exactly this on a college campus as it is a completely different setting. In this case, the student provided a vulgar speech in front of a large crowd in an assembly. A forum like this would not be too common on a college campus usually because of the way college is structured. A college student likely would not be reprimanded like in this case because college is a more adult setting than a high school.

At the University of Missouri, a twenty-two-year-old graduate school student was involved in an underground school newspaper. She decided to do a piece that was an allegedly indecent political cartoon. The cartoon in question depicted a police officer sexually assaulting the Statue of Liberty. The University, in response to that publication, expelled Papish. The University requires that their students uphold a certain level of decency and prohibit speech that could be classified as indecent according to the university's General Standards of Student Conduct. Papish was allowed to stay on campus until the semester concluded but she was not given credit for one of the classes she passed. The lower courts involved prior to the United States Supreme Court upheld the expulsion of Papish. The Supreme Court determined the University of Missouri was wrong to expel the student for her publication as it is a violation of her free speech rights. The Court also determined that the university's action was not justified, and the university must give

Papish the credit she has earned for the class. Regarding the dissemination of ideas the Court deciding this case said, "We think *Healy* makes it clear that the mere dissemination of ideas -- no matter how offensive to good taste -- on a state university campus may not be shut off in the name alone of "conventions of decency."²⁰ The Court essentially made it clear that banning speech on the basis of decency is a difficult thing to do because there are several protections. Beyond that the Court also touched on the publication aspect of this case in saying, "[It's] clear that neither the political cartoon nor the headline story involved in this case can be labeled as constitutionally obscene or otherwise unprotected."²¹ This precedent was found in *Cohen v. California* (1971) among other cases.

Discussion with other Publications

In *Free Speech on Campus* written by law professors Erwin Chemerinsky and Howard Gillman, they address this exact issue on a specific page. The authors talk about what the public universities can and cannot do to limit speech of their students. In the section they mention *Frisby v. Schultz* (1988) in the paragraph prior to this quote. The authors say, "but even regulations meant to protect a captive audience cannot be based on the ideas espoused. What is placed on walls or bulletin boards or in dormitory windows may be regulated so long as the rules are content neutral and applied in a content-neutral manner"²². This sets up a university to ban whatever they choose within reason. By content neutral the authors mean, "A campus could have a rule preventing students from affixing anything to the windows of their dormitory rooms, but a campus could not prohibit just the display of Confederate flags on dormitory windows"²³. These quotes are almost in succession of each other. Basically, since dormitories are considered private forums and there could be a captive audience, there can be more limitations to speech granted it is content neutral.

I take issue with some things said by these authors. The first thing that I take issue with is the idea of a dorm building being a captive audience. The definition of a captive audience is, "a person or people who are unable to leave a place and are thus forced to listen to what is being said" according to the Merriam Dictionary. That is not at all the way I see the dormitory setting. The authors Chemerinsky and Gillman referenced *Frisby v. Schultz* (1988). The *Frisby* case has totally different circumstances surrounding it. The parties involved were Sandra Frisby and Robert Braun. Frisby and Braun gathered several like-minded people to stand outside of the home of a doctor who performed abortions. This group of people picketed outside of his home making it difficult for him to leave his house. The United States' Supreme Court determined that the city's ordinance outlawing picketing outside of residential homes was constitutional. The Court held that the ban was indeed content neutral, served a significant government interest, and is narrowly tailored enough to allow for adequate alternatives.²⁴ When the Court describes a law that is narrowly tailored, they mean a law that allows for other channels to be left open for ideas to be expressed. In this case, the interest is to prevent a captive audience. All three of these criterion must be met to meet the intermediate level of scrutiny that this case calls for. In this case, the Court determined that all three items were satisfied. Now, comparing *Frisby v. Schultz* (1988) to the dorm window display ban that the authors describe, they appear very different. It starts to blur the line of what really is a captive audience. A captive audience is someone who is forced to listen to what someone else has to say. When comparing the hypothetical case from the authors and *Frisby v. Schultz*, the definitions of a captive audience seem completely different. I would argue that saying a captive audience extending to members of a dormitory or residence hall is stretch.

The authors pointed out *Frisby v. Schultz* (1988) in particular, however there is a case that is much more suited to use. The case I think would be more appropriate to use is as it pertains to window signage specifically would be *City of Ladue v. Gilleo* (1994). In this case the City of Ladue implemented an ordinance that banned signage as an effort to minimize clutter. This ordinance was determined to be not content neutral as there was other signage that was permitted. Gilleo placed signs in her yard and the sign was knocked over. The police said she could not place a sign there due to a city ordinance which was intended to minimize clutter. She filed a petition then challenged the law even further by putting a sign in the window of home. The United States Supreme Court held that the city's ordinance has a significant government interest but fails in content neutrality as well as leaving ample channels open. In the Court decision written by Justice Stevens the Court said, "A special respect for individual liberty in the home has long been part of our culture and our law; that principle has special resonance when the government seeks to constrain a person's ability to speak there."²⁵ In this quote the Court determined that there is a special respect given to people and their right to speech in their homes. Additionally, it could be said that someone's home is a private forum and not a traditional public forum like it was in *Frisby v. Schultz* (1988).

In the journal article, *Walls, Halls and Doors: First Amendment Issues for Public Spaces in Housing*, authors Tess Barker and Amanda McLittle discuss many issues that are currently relevant in the public university setting. In their publication they directly mention externally-facing windows. Barker and McLittle determined "exterior facing windows are not a default traditional public forum and the institution can impose standards and limits"²⁶ Later in that section they say, "If the housing contract already does not have this existing policy, the staff cannot ask residents to take down a flag, unless it is a fire hazard, even if others find it offensive or distasteful."²⁷ The authors then add their opinion on the Captive Audience Doctrine. They say, "Once the institution allows residents to use their doors, windows, or other forum for expression, it cannot exclude the resident who wishes to use that forum to express offensive but protected speech."²⁸ According to this, some of the university policies at the beginning of this paper are potentially problematic.

Another important topic of discussion is the notion that universities are not allowing for nearly as much speech as they previously did. Many universities have policies for reporting bias incidents. In a letter from the University of Chicago's Dean Ellison, he said, "Bias incidents... include any actions that are motivated by bias, even if they do not include the elements required to prove a hate crime or a violation of University policy."²⁹ This is not to say that this is bad policy, this can help deal with students feeling intimidated. When this policy continues to go further, that is when it becomes too restrictive. Greg Lukianoff, the author of *Unlearning Liberty: Campus Censorship and the End of American Debate*, said, "Administrators have been able to convince well-meaning students to accept outright censorship by creating the impression that freedom of speech is somehow the enemy of social progress"³⁰ The sentiments of this author are strong and may go too far, but it relates to universities implementing policies restricting free speech.

Conclusion

So, is a university's ban on window displays constitutional? It depends on several factors. The restriction must prove to be content neutral, leave other channels open, and serve a significant government interest. In the cases of late involving universities, they appear to be content neutral for the most part. The part that could become an issue is if a student challenges whether there are ample channels of expression still available or if there is a significant government interest being met. I think that it would be wise to yield to the *City of Ladue v. Gilleo* (1994) case. In that case

it was determined that reducing city clutter was a significant government interest. However, there were not ample alternatives, and it was not content neutral. Many campus enacting regulations such as the display bans have been wise enough to make their ban content neutral. The problems that lie ahead are whether the “significant interest” the public university is citing is actually significant. One could assume that if clutter to a city is significant enough, damage to university property or safety concerns would also be covered. If this ever is taken to the United States Supreme Court, the Court may want to look at what was said in *Ladue* (1994), “A special respect for individual liberty in the home has long been part of our culture and our law; that principle has special resonance when the government seeks to constrain a person’s ability to speak there”. This begs the question, does a residence hall dorm room classify as a home. Throughout my time on a college campus, I have always heard university officials refer to the residence halls as our homes regardless of the campus I visited. If that is the case, shouldn’t there be a special respect held for students to display their views from the window of their home (dorm window). This creates an interesting wrinkle in things that leaves much to be desired for students in the future.

One thing can be inferred from this research though. Some university policies are content neutral like Ohio State University, UW-La Crosse, and UW-Eau Claire’s policies. There is a claim to be made that these universities are not offering ample channels to express themselves. For instance, when you look at UW-Eau Claire, you will notice that the policy is restrictive of what students can put in their windows. This does allow for them to post material in their rooms, just not in the window. There is nothing in the policy that would prohibit other forms of expression though. Examples of expression that are permitted by the policy are oral speech, or any of the other forms mentioned in cases mentioned in this paper. There are several channels for students to use their rights, they just may not be as passive or convenient as a window display. Continuing the discussion about UW-Eau Claire, there are limited channels open on lower campus such as areas you must reserve there are not many known areas in which students can express themselves with speech or protest. Despite there being several channels available to students who wish to use their free speech, they may just be uninformed of the methods they can. In contrast to UW-Eau Claire and UW-La Crosse, other policies are not content neutral, like UW-Green Bay, UW-Parkside, UW-River Falls, and UW-Platteville. The policy at UW-Green Bay is potentially problematic because the speech they are attempting to discourage is speech that is intended to create an intimidating environment. This is not content neutral. UW-Parkside’s policy is attempting to limit speech that harasses a specific group of people. This is also not a content neutral rule. At UW-River Falls, the speech they are attempting to restrict is the speech that targets specific people or groups of people. This is not content neutral either. Finally, UW-Platteville’s policy restricts what students can put in windows to twinkle lights around the window. This is potentially not content neutral because it is not all or nothing. The previous four universities potentially fail the content neutrality portion of immediate scrutiny. The two universities mentioned before them could fail to meet the part of the test stating the rule must be narrowly tailored enough to allow for ample other channels. For the moment, what my research shows is that there are several universities policies around the UW System that are not as content neutral as they need to, and others may violate the narrowly tailored portion of the immediate scrutiny.

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Structural and Energetic Properties of OC–BX₃ Complexes: Unrealized Potential for Bond-Stretch Isomerism

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Abstract

This project explored the structural and energetic properties of OC–BX₃ (X = F, Cl, or Br) complexes using computations and low-temperature infrared spectroscopy. Calculations provided equilibrium structures, binding energies, vibrational frequencies, and B–C potential energy curves. The OC–BF₃ system is a weak, long-bonded complex with a single minimum on the B–C potential ($R_{\text{B-C}} = 2.865 \text{ \AA}$). For the remaining two complexes, OC–BCl₃ and OC–BBr₃, computations predicted two stable minima on their B–C potential curves. The BCl₃ system is a weak complex with a long bond ($R_{\text{B-C}} = 3.358 \text{ \AA}$), exhibiting a secondary minimum with a short bond length of 1.659 Å. For OC–BBr₃, the system is a weak complex with a short bond at 1.604 Å, and a secondary minimum with a long bond length ($R_{\text{B-C}} = 3.483 \text{ \AA}$). Matrix-isolation experiments showed evidence of the long-bond form of the BCl₃ and BBr₃ complexes, while in the presence of HX contamination, but no evidence of the short-bond form. Peak assignments were made in the CO region via comparison to predicted frequency shifts. Two peak assignments were made in the BCl₃ complex (2145 cm⁻¹ and 2154 cm⁻¹), and assignments were made for the BBr₃ complex (2143 cm⁻¹ and 2151 cm⁻¹). The gases (CO and BX₃) proved to be unreactive when mixed at room temperature.

Introduction

Interest in molecular complexes and their structural and energetic properties has continued for years since the 1960's. The term “molecular complex” refers to any association of two otherwise stable molecules, and are also known as “charge-transfer” complexes, “donor-acceptor” complexes or “Lewis acid-base” complexes. These all refer to some degree of electron transfer between the two components, and thus have a narrower definition than the term “molecular complex.” In any event, each constituent in one of these interactions has a specific role. A “donor” or “Lewis base” has a lone pair of electrons which it can donate to an acceptor, and an “acceptor” or “Lewis acid” has a vacant bonding site to accommodate a lone pair of electrons. One reason for continued interest in such complexes stems from on-going computational chemistry research, as molecular complexes serve as energetic benchmarks via the “Charge Transfer Database.” Developers of various density functional theory (DFT) methods such as M06 and M06-2X have used the “Charge Transfer Database” to verify the energy predictions of these methods. Computations have also identified a continuum in bonding going from van der Waals interactions to covalent bonds, which has been illustrated in comprehensive, comparative studies. These studies found that no one feature (e.g. electrostatics, charge-transfer, etc.) contributed a fixed amount to any system or paralleled strength in any consistent, systematic manner. In addition, computations have led to a further redefining and classification of “molecular complexes,” which took place recently with terms having narrower definitions

in reference to the geometries about the acceptor center, specifically “ σ -hole” and “ π -hole” interactions. A σ -hole is like a sigma bond, in that the electron deficient region of the acceptor has cylindrical symmetry about the bond, or in other words, the electron deficient region is in line with the bond.^{4,5,6} This region (a positive σ -hole) can interact with a negatively charged region of a donor atom, such as a lone pair of electrons. Some examples of σ -hole interactions include hydrogen bonding,^{4,5} halogen bonding,^{4,5} and MX_4 coordinate bonds.⁶ By contrast, a π -hole exhibits two regions of electron deficiency which extend perpendicular to the molecular framework (i.e., above and below the plane of the bond). The most common examples of π -holes are MX_3 acceptors such as BCl_3 .

Another reason for continued interest is the fact that some molecular complexes undergo major changes in structure when going from the gas phase to the solid state or other stabilizing, condensed-phase environments. Perhaps the most vivid examples are the nitrile- BF_3 complexes, which are also π -hole complexes, and undergo major structural changes in condensed phases. The specific signature of this effect is a contraction of the B-N distance and a distortion of the acceptor molecule, BF_3 in this case. The clearest examples of this are gas-solid structure differences, which tend to occur in systems with intermediate degrees of bonding (i.e. when the length is between that of a non-bonded, van der Waals interaction, and a fully covalent bond). One of the examples of a gas-solid structure difference is the $\text{CH}_3\text{CN}-\text{BF}_3$ system, which has been previously studied via X-ray diffraction and microwave spectroscopy experiments.⁹ In the gas phase, $\text{CH}_3\text{CN}-\text{BF}_3$ has a B-N distance of 2.011 Å, which contracts by 0.381 Å in the solid state, to a distance of 1.630 Å.⁹ The F-B-N angle in the gas phase is 95.6°, which distorts to 105.6° in the solid state, a difference of 10°. Further studies on the analogous systems from the Phillips group led to the study of $\text{FCH}_2\text{CN}-\text{BF}_3$ and $\text{ClCH}_2\text{CN}-\text{BF}_3$, finding even more extreme structure differences, B-N distances contracting by 0.7 Å for either complex, and F-B-N angle distortion of at least 11° in the solid state. The differences in structure from the gas phase to solid state have also been studied in other systems such as $\text{HCN}-\text{BF}_3$, NH_3-SO_3 , and amine- SO_2 complexes.

The Phillips group has demonstrated that significant changes in structure can be induced by even inert media such as noble gas matrices, and have proposed a generalized mechanism for this effect. The signatures of these effects are shifts in the acceptor modes in the infrared (IR) spectrum of the complex (relative to the free acceptor). The nitrile- BF_3 systems have been shown to parallel the B-N distance and this reflects a progressive contraction of the B-N bond. Scans of the potential energy curve noted a distinct shape in medium-sensitive systems, specifically, a long equilibrium distance and a gentle rise along the inner wall of the curve. In dielectric media, the inner region stabilizes, such that the minimum shifts inwards with solvent stabilization. These models of the effect on the potential predict the observed contraction for $\text{XCH}_2\text{CN}-\text{BF}_3$. In an extreme case, similar models predict a 1.0 Å shortening for $\text{CH}_3\text{CN}-\text{SiF}_4$,¹⁷ from 3.0 Å in the gas phase to 2.0 Å in a dielectric media of $\epsilon = 20$.

In subsequent studies of the $\text{CH}_3\text{CN}-\text{BCl}_3$ complex, the Phillips lab observed a different, novel aspect of the intermolecular potential – the occurrence of two distinct minima along the B-N coordinate. These minima, corresponding to so called “short-” and “long-bond” structures, is an example of a phenomenon known as “bond-stretch isomerism.” This has been a contentious topic since the 1990’s, after a paper by Gerard Parkin came out refuting a previous paper that claimed to have experimental evidence of such isomers. It was later seen that the experimental data from the previous paper had been contaminated, leading to the conclusion that there were two distinct structures with differing bond lengths.¹⁹ Since then, the idea of bond-stretch isomers has been dismissed as a myth by most due to the lack of experimental evidence. Despite this,

computational results on $\text{CH}_3\text{CN}-\text{BCl}_3$ predicted two distinct minima (short bond structure: 1.601 Å and long bond structure: 2.687 Å),¹⁸ separated by a significant barrier, which persisted at high-levels of theory (CCSD/aug-cc-pVTZ). In turn, the Phillips group conducted low-temperature, matrix-isolation IR experiments in an effort to isolate and characterize these distinct structures. However, they only observed signals due to the “short-bond” form, while overlapping signals on the IR spectra in the CH_3CN region did not allow for confident identification of peaks due to the long-bond structure. The overlap problem stems from the fact that BCl_3 decomposes into HCl in any trace amounts of water, which forms a complex ($\text{CH}_3\text{CN}-\text{HCl}$) that shifts to a similar degree as the C–N stretching mode of the long-bond form for the $\text{CH}_3\text{CN}-\text{BCl}_3$ complex. In the BCl_3 region, the shift for the long-bond form is unlikely to have moved the band beyond the broad signals of free BCl_3 .

Following the study of $\text{CH}_3\text{CN}-\text{BCl}_3$, and finding that the short-bond form of the complex was much lower in energy, the group sought to weaken the donor-acceptor bond by adding halogens to the nitrile, which would presumably place the short and long minima at more equal energies. For $\text{FCH}_2\text{CN}-\text{BCl}_3$ and $\text{ClCH}_2\text{CN}-\text{BCl}_3$, they found that the addition of halogens did weaken the bond; the binding energies of the short-bond forms were 5.3 kcal/mol and 6.3 kcal/mol for $\text{FCH}_2\text{CN}-\text{BCl}_3$ and $\text{ClCH}_2\text{CN}-\text{BCl}_3$, respectively. In addition, the energy of the long-bond forms (3.2 kcal/mol and 3.3 kcal/mol) were much less affected, thus the two forms were much closer in energy than the two $\text{CH}_3\text{CN}-\text{BCl}_3$ forms. In spite of the effect of halogen substitution, which did somewhat equalize the energies of the two minima, on the potential, they again only observed the short-bond form in matrix-IR experiments.²¹

Here, we extend this work to similar systems for which there are some advantageous features. Carbon monoxide (CO) is isovalent with nitriles, but is a much simpler base and only has one frequency in a sparse region of the spectrum, which would facilitate the observation of distinct, but often slight, frequency shifts. This manuscript will report the structural and energetic properties of $\text{OC}-\text{BX}_3$ ($X = \text{F}, \text{Cl}$ or Br) complexes, including equilibrium structures, predicted gas-phase frequencies, and potential energy curves, via quantum-chemical computations and IR experiments in Ar and Ne matrices. The BCl_3 and BBr_3 complexes with CO both show potential for the observation of distinct structures with different B–C minima, particularly in the latter case. By contrast, $\text{OC}-\text{BF}_3$ is a weakly-bonded system with a known experimental structure that provided a valuable computational benchmark.

Materials and Methodology

Computations

Post Hartree-Fock and DFT calculations were performed using Gaussian09 B.01 and Gaussian16 C.01. Symmetry was constrained to C_{3v} geometry throughout these computational studies. Optimizations were performed with convergence criteria set using the “opt=tight” option, and an ultrafine grid was employed for all DFT and MP2 calculations.

Overall, we utilized multiple DFT methods (M05, M06, M06-2X, $\omega\text{B97X-D}$, M08-HX, M11, MN12-SX, and MN15) along with MP2 and CCSD with the aug-cc-pVTZ basis set. A validation study was conducted, as in previous work by the group, which was based on acceptor frequencies and experimental gas-phase structures.^{12, 18} The structures and energies reported here are from $\omega\text{B97X-D}$, which had the smallest RMS error when predicting experimental BF_3 and BCl_3 frequencies (7.23 cm^{-1} and 8.75 cm^{-1} for BF_3 and BCl_3 respectively). In addition, $\omega\text{B97X-D}$

also most accurately predicted the experimental structure of OC–BF₃ (0.023 Å difference in B–C distance). However, we do note that M06 was somewhat more accurate in predicting BBr₃ frequencies (RMS_{M06} = 3.2 cm⁻¹ and RMS_{ωB97X-D} = 13.5 cm⁻¹). Calculations of the B–C potential were conducted in a pointwise manner from 1.4 Å to 3.0 Å in steps of 0.1 Å using the following methods: M05, M06, M06-2X, ωB97X-D, MP2, and CCSD, all with the aug-cc-pVTZ basis set. The energies on the CCSD curves were based on MP2 geometries. Among these methods, ωB97X-D most closely matched the CCSD energies along these curves, but most methods—aside from MP2—were qualitatively similar. It should be noted that while we did validate on acceptor modes, we ended up analyzing peaks in the CO region only; however, the methods (M05, M06, M06-2X, ωB97X-D, and MP2) poorly predict the CO peak with the closest prediction having an RMS value of 31 cm⁻¹. Validation on acceptor modes were therefore warranted due to its better predictions and the fact that we used relative shifts (complex frequency – free CO) when identifying experimental peaks.

Materials

CO (99.0%) was obtained from Sigma-Aldrich and used without further purification. BCl₃ (99.9%) was obtained from Sigma-Aldrich and was transferred to a sample tube by filling a 2L bulb to a pressure of 1-2 atm, and then condensing in a 50mL sample tube with liquid nitrogen. Further purifications of the BCl₃ sample was completed before the final experimental sample was obtained. Purification of BCl₃ was achieved through a two-step process: first freeze-pump-thaw cycles at the temperature of liquid nitrogen (77K), then the sample was subjected to active vacuum in several 10-30 second intervals at 183K via a liquid nitrogen/methanol bath. The BBr₃ was obtained at a purity of 99.995% from Sigma-Aldrich, transferred to a sample tube via a disposable pipet, and was further purified by freeze-pump-thaw cycles in a liquid nitrogen bath at 77K. Ar (99.9999%) and Ne (99.999%) were obtained from Praxair and used with no further purification before use.

Matrix Isolation IR Spectra

IR spectra were collected using two previously described matrix-isolation apparatuses. Initial spectra in argon matrices were collected using a ~10K system that utilizes a Cryomech ST15 optical cryostat and a Nicolet Avatar iS20 FTIR spectrometer at 1.0 cm⁻¹, averaging 400 scans per spectrum. Matrix samples were deposited on a KBr window, and the temperature was maintained using a Scientific Instruments 9600-1 temperature controller with a single Si diode located at the end of the second refrigeration stage. The majority of experiments were conducted in neon matrices on a 4K system¹⁸, that employs a Janis SHI-4–5 optical cryostat and a Thermo Nexus 670 FTIR. The resolution was 1.0 cm⁻¹, and 400 scans were averaged for each spectrum. Matrix samples were deposited onto a gold mirror and spectra were collected by reflecting the IR beam off the sample mirror (~45°) and onto an external (DTGS) detector. Samples were prepared in 2L glass bulbs on a glass manifold (Chemglass) evacuated with a diffusion pump (Chemglass) by making separate gas mixtures containing either CO or BX₃ (X = Cl or Br) in Ar or Ne. For experiments, matrix samples were deposited by simultaneous flow of gas mixtures in separate lines that merge immediately before the mirror. A final set of experiments using single mixtures containing both hosts in the same bulb and deposition line yielded identical spectra. Mixtures were deposited at temperatures ranging from 3.5K to 6K and most experiments ran two 60-minute depositions after which most samples were annealed at temperatures of 8-9K for 30

minutes to one hour with spectra recorded after each deposition and anneal. Flow rates typically ranged from 1-10 mmol/hr with mixture concentrations ranging from 1/32%-1/2%. It should be noted that faster deposition rates seemed to reduce the amount of HX in the spectrum, relative to BX_3 . This suggests decomposition in the deposition plumbing, in spite of the fact that the lines were mainly Teflon, with as little metal plumbing as possible.¹⁸

Reactivity experiment

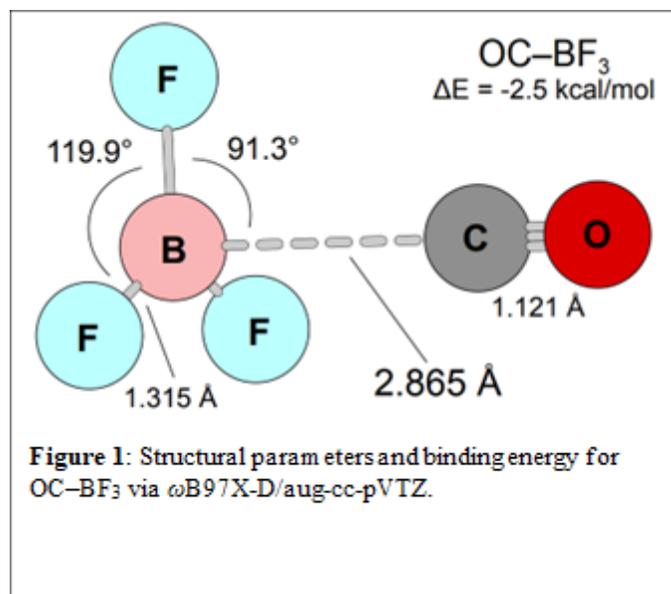
To ensure that we could perform the single-bulb experiments described above we examined the direct reaction of CO and BCl_3 or BBr_3 . ~50 torr each of BBr_3 and CO were introduced into a 100mL reaction flask on our preparatory manifold. The gases were allowed to mix for ~20 min before sitting in an ice bath for ~10 min. Neither of these produced a visible reaction product.

Results and Discussion

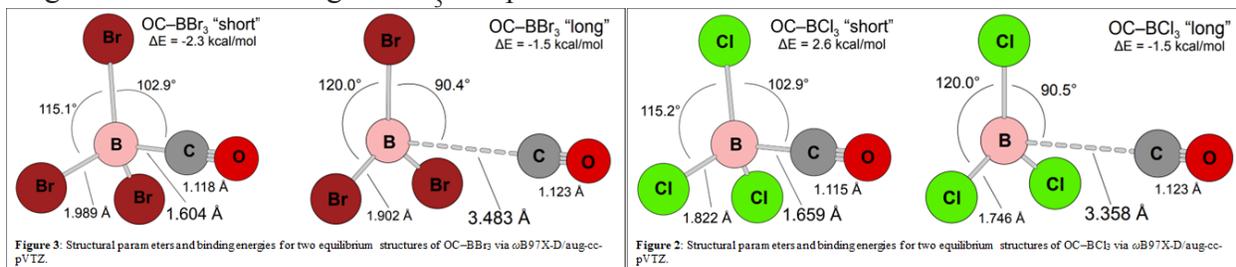
Equilibrium Structures

Due to previous work on $CH_3CN-BCl_3$ ¹⁸ and $XCH_2CN-BCl_3$,²¹ we expected two distinct structures for the Cl- and Br-containing complexes with different B–C distances, so when searching for equilibrium structures we started the optimizations from two different geometries with short (1.6 Å) and long (3.0 Å) B–C distances. As noted above, we used a variety of DTF methods as well as MP2 and CCSD in our calculations, settling on ω B97X-D for most of the analysis in this manuscript. All other methods, as noted above, produce relatively consistent results.

All structural parameters for $OC-BF_3$ are shown in Figure 1. This complex has a single, distinct structure with a long B–C distance (2.865 Å) and is thus a weakly-bonded system with a binding energy of 2.5 kcal/mol. These results are consistent with the recently updated structure of $OC-BF_3$,²⁵ which puts the B–C distance at 2.888 Å. For context, the van der Waals radii for carbon (1.7 Å) and boron (1.8 Å) add to a sum of 3.5 Å, 0.6 Å longer than the B–C distance of $OC-BF_3$, while the sum of the covalent radii for carbon (0.77 Å) and boron (0.84 Å) is 1.61 Å, significantly shorter than the calculated B–C distance of 2.865 Å. The acceptor molecule (BF_3) has little distortion from its trigonal planar geometry. The F–B–C angle is 91.3°. Compared to a similar, isoelectronic complex, specifically $HCN-BF_3$,² the $OC-BF_3$ complex has a considerably longer donor-acceptor distance, with $HCN-BF_3$ having an experimental B–N distance of 2.473 Å, almost an angstrom shorter than the van der Waals radii. However, the F–B–N angle is larger in the HCN complex (93.9°); the BF_3 is slightly more distorted compared to the $OC-BF_3$ complex. The binding energy of the HCN complex is 5.0 kcal/mol via ω B97X-D/aug-cc-pVTZ, which makes the complex twice as strong as the analogous CO complex, which has a binding energy of 2.5 kcal/mol.



We do predict two structures for the OC-BCl_3 and OC-BBr_3 complexes, which we refer to as the “short-” and “long-bond” forms. The long-bond forms of OC-BCl_3 and OC-BBr_3 are similar to the OC-BF_3 complex, all being weakly bonded systems with little distortion in the acceptor molecule. Both complexes have slightly longer B-C distances than the BF_3 complex, 3.358 Å and 3.485 Å, respectively, much closer to the sum of the carbon and boron van der Waals radii than the BF_3 complex. Both complexes exhibit similar X-B-X and X-B-C angles with the former being 120°, and the latter being 90.5° and 90.4° respectively, slightly less distorted than OC-BF_3 . A complete set of structural parameters are included in Figures 2 and 3. Binding energies for the Cl- and Br-containing complexes are both 1.5 kcal/mol, which is 1.0 kcal/mol smaller in magnitude than the analogous BF_3 complex.



These long-bond structures resemble their analogs in the nitrile- BCl_3 systems, specifically $\text{CH}_3\text{CN-BCl}_3$.¹⁸ Again, while the donor-acceptor distances cannot be directly compared due to differences in the atoms of the interaction, an indirect comparison can be used. For $\text{CH}_3\text{CN-BCl}_3$, the predicted MP2/aug-cc-pVTZ B-N distance is 2.687 Å, 0.663 Å shorter than the sum of the van der Waals radii, while the B-C distances for the OC-BCl_3 and OC-BBr_3 forms differ by only 0.142 Å and 0.015 Å respectively. This points to a slightly weaker interaction in the long-bond form of the OC-BX_3 complexes compared to $\text{CH}_3\text{CN-BCl}_3$. This observation is substantiated by the values of the binding energies. Having a $\Delta E = -4.9$ kcal/mol, $\text{CH}_3\text{CN-BCl}_3$ is almost 3.5 kcal/mol larger in magnitude than the CO complexes, which both have binding energies of 1.5 kcal/mol. However, overall, all these systems are fairly weak complexes, showing little distortion in the acceptor molecule, with long donor-acceptor distances approaching the

sum of their respective van der Waals interaction radii.

Turning now to the short-bond structures for both the Cl- and Br-complexes, we found both systems exhibit large distortions of the acceptor molecule due to the short B–C distance. For OC–BCl₃, the B–C distance is 1.659 Å, which is 0.055 Å longer than OC–BBr₃ ($R_{B-C} = 1.604$ Å). Interestingly, both B–C distances are close to the sum of the covalent radii of boron (0.84 Å) and carbon (0.77 Å) which is 1.61 Å, another indication that the short-bond forms of these complexes are genuinely bonded. The X–B–C angles are both 102.9°. The binding energies of these structures, unlike their long-bond counterparts, are not similar with OC–BCl₃ at $\Delta E = +2.5$ kcal/mol (i.e., above the separated fragments), while the OC–BBr₃ complex is closer to its long-bond form ($\Delta E = -2.3$ kcal/mol), about 0.8 kcal/mol lower in energy. Energetically these complexes resemble N₂–BH₃ – in which there is a very short B–N distance, but the binding energy is fairly small – offset by repulsive interactions between donor and acceptor.

Structurally, these short bond forms resemble the analogous structure of the CH₃CN–BCl₃ system.¹⁸ The B–N distance is 1.601 Å, this time longer than the sum of the covalent radii by 0.051 Å. The OC–BCl₃ complex has a donor-acceptor distance that is also longer than the sum of the covalent radii (0.049 Å), while OC–BBr₃ is 0.006 Å shorter than the covalent radii. The distortion of the Cl–B–N angle is almost 2° larger than the analogous angle in the OC–BX₃ complexes, and the binding energy of the nitrile complex is again lower in energy, all pointing to a stronger interaction of the CH₃CN–BCl₃ complex compared to OC–BX₃. For a more classic example, we turn to H₃N–BCl₃.² The B–N distance is 1.618 Å, again just longer than the sum of the covalent radii (0.068 Å). The binding energy for the ammonia complex is significantly larger in magnitude than those of the CO complexes, at 28.2 kcal/mol (MP2/6-31+G(2d,p)//MP2/6-31+G(2d,p)).³¹ Compared to the acetonitrile short-bond form, the ammonia complex is a longer bond and stronger interaction. It should be noted that the Phillips group has seen this phenomenon in RCN–BH₃ complexes, which show that nitriles have shorter B–N bonds and a smaller $|\Delta E|$ compared to amines. The ammonia molecule has a lone pair of electrons which has sp³ hybridization, which is more extended in space than the sp lone pair, allowing for overlap to occur at a longer distance. The sp lone pair of the CH₃CN molecule extends to a lesser degree than that of the ammonia molecule, which must be closer to the acceptor molecule for overlap to happen. The π electrons of the C–N triple bond and the halogens of the acceptor molecule also repel each other, which (as noted above for N₂–BH₃) raises the overall energy and covalent bonding, making for a shorter and weaker interaction in the CH₃CN complex. Overall, these complexes are structurally indicative of covalent or charge transfer interactions with short donor-acceptor distances close to the sum of their covalent radii that result in large distortions of the acceptor molecules. However, energetically these complexes are fairly weak interactions.

Donor-Acceptor Potentials

Figures 4 through 6 display the boron–carbon potential energy curves computed via a variety of DFT methods along with MP2 and CCSD with the aug-cc-pVTZ basis set. These curves offer two major observations; one being the relative energy between the two minima of the Cl- and Br-containing complexes and the other being the barrier that exists between the two structures. It should be noted that the OC–BF₃ curve resembles that of systems prone to condensed phase media effects, with its relatively long minimum and gentle rise towards the inner portion of

the curve.⁸ However, the system has been studied experimentally and shows only the slightest indication of a matrix-induced contraction of the B–C bond. Previously conducted matrix isolation experiments of CO/BF₃ assign complex peaks at 1438.7/1437.3 cm⁻¹ and 1441 cm⁻¹ for argon matrices.^{33,34} We predict a frequency at 1444 cm⁻¹ (ω B97X-D/aug-cc-pVTZ), in reasonable agreement with experimental frequencies. However, the 3-7 cm⁻¹ shift between the matrix data and the predictions is consistent with a slight compression of the B–C bond. For OC–BCl₃, the energy maximum occurs around 2.1 Å (+5.9 kcal/mol) with the energy +7.4 kcal/mol relative to the outer minimum, and the two minima are 4.1 kcal/mol apart. For OC–BBr₃, the barrier occurs at 2.2 Å (5.7 kcal/mol) with the energy +7.2 kcal/mol relative to the outer minimum. The two minima are closer in energy to each other than those of the BCl₃ complex with a difference of only 0.8 kcal/mol between them. It should be noted that CCSD seems to prefer a slightly higher energy on the inner part of the curve, which may explain why we are unable to see two distinct structures in experiments (see discussion below), despite what we predict computationally.

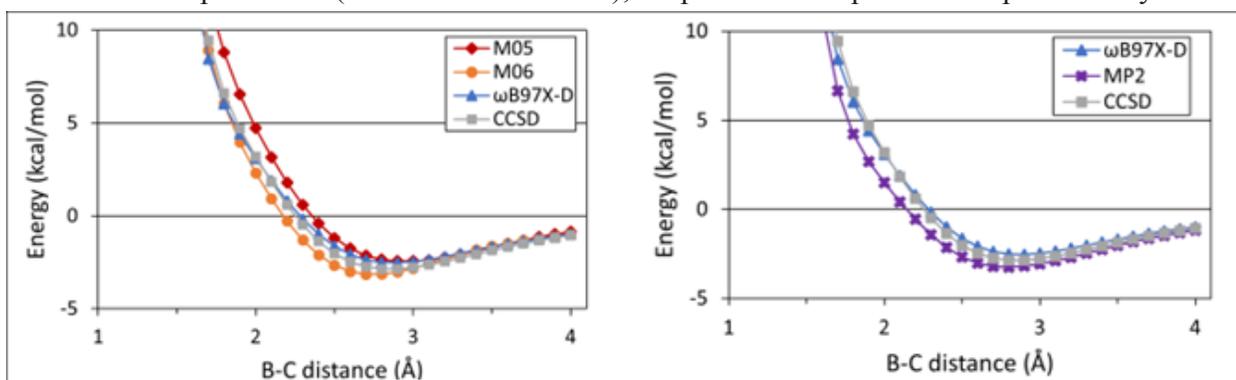


Figure 4: Calculated B–C bond potentials for OC–BF₃ using hybrid DFT methods (and CCSD) with the aug-cc-pVTZ basis set (left) and post-HF methods and ω B97X-D with the aug-cc-pVTZ basis set (right). An M06-2X/aug-cc-pVTZ curve was calculated but was not included because it is essentially superimposed on the M06 curve.

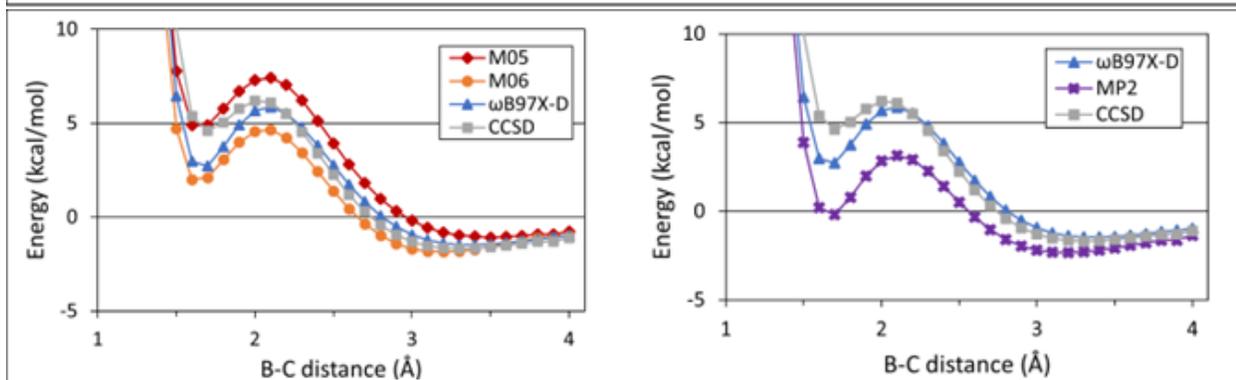


Figure 5: Calculated B–C bond potentials for OC–BCl₃ using hybrid DFT methods (and CCSD) with the aug-cc-pVTZ basis set (left) and post-HF methods and ω B97X-D with the aug-cc-pVTZ basis set (right). An M06-2X/aug-cc-pVTZ curve was calculated but was not included because it is essentially superimposed on the M06 curve.

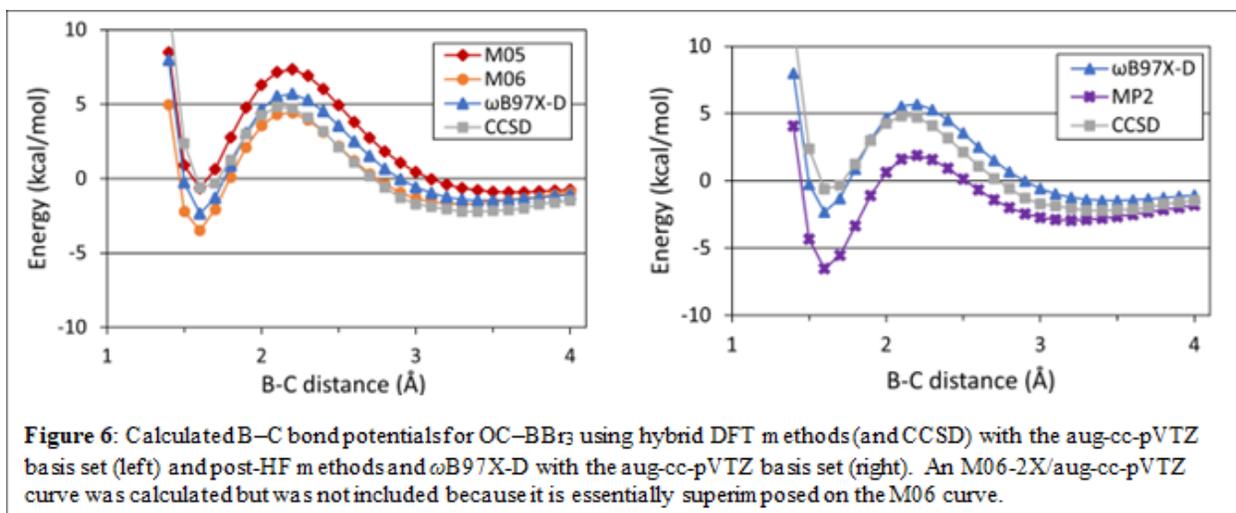
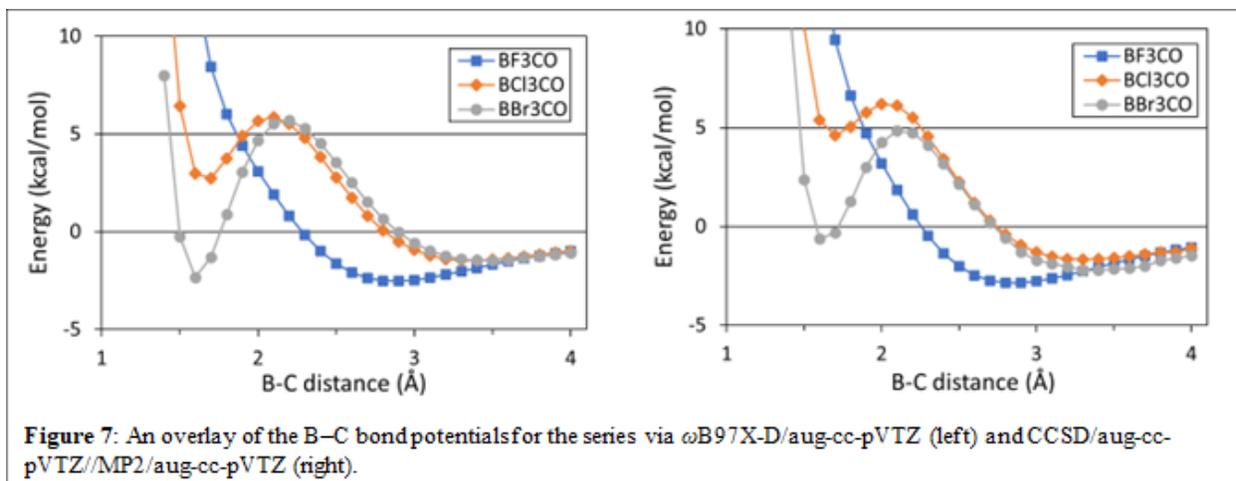


Figure 7 shows two different overlays for the three different complexes, one for the ωB97X-D and another for the CCSD potential curves, to facilitate comparison between the shapes of each curve. The graphs not only display the clear difference in energy between the two minima for the BCl₃ and BBr₃ complexes; but also, the difference in the calculated energies between DFT and CCSD. For ωB97X-D, the calculations predict a much lower-energy inner minimum for BBr₃ with a higher energy outer minimum, while CCSD does the exact opposite, predicting a higher energy inner minimum and a lower energy outer minimum. As for the BF₃ complex, CCSD predicts a much steeper rise towards the inner wall than ωB97X-D, while both predict the minimum at relatively the same B–C distance, 2.9 Å and 2.8 Å for ωB97X-D and CCSD respectively. Another observation these graphs lend themselves to is an explanation as to why BF₃ does not show two distinct structures like the BCl₃ and BBr₃ complexes. Overall, this situation mimics what the group has seen previously for nitrile–BX₃ complexes and rationalized via energy decomposition analyses. The barrier results from repulsion between the larger halogens (Br or Cl) and the π electrons on C–N or C–O. These are ultimately offset by bonding interactions (more or less) at short distances. For BF₃ systems, the favorable bonding interactions and π-halogen repulsions set in at similar distances, rendering the potentials flat (and leading to medium effects for nitrile–BF₃ systems). Lastly, the overall trends in the energies in the short- and long-bond regions of the curve can be rationalized in terms of key acceptor properties (charge in the boron atom and LUMO energies). The energy in the short bond region parallels LUMO energies (BBr₃ < BCl₃ < BF₃), which one might expect to correlate with stronger bonding interactions (BBr₃ > BCl₃ > BF₃). On the other hand, the charge on the central atom is greatest on BF₃, and this seems consistent with OC–BF₃ having the lowest energy minimum in the long bond region (though arguably this effect is convoluted with the onset of repulsion for BCl₃ and BBr₃).

Acceptor molecule	q _B (eV)	ELUMO (kcal/mol)
BF ₃	1.4	0.04
BCl ₃	0.34	0.002
BBr ₃	0.064	-0.01

Table 1: Summary of boron charges and LUMO energies



IR Spectra and Frequencies

We recorded IR spectra of complexes in low-temperature matrices, focusing mainly on the neon matrix although we did some experiments with CO/BCl₃ in argon as well. For both OC–BCl₃ and OC–BBr₃ we were able to assign one band in the CO region to an OC–BX₃ complex guided by predicted frequency shifts using ω B97X-D. Figures 8 and 9 show the spectra for both complexes in neon. In the OC–BCl₃ complex, we see two peaks in the complex spectra that do not appear in the CO reference spectrum. Of these, we assigned the peak at 2144 cm⁻¹ to the long-bond form of the complex, while the peak at 2154 cm⁻¹ is due to the HCl impurity forming a complex with CO. While specific frequency predictions agree only marginally with what is observed in experiments (we validated on acceptor modes—see discussion above), calculated shifts (complex – free molecule) are consistent with observations. For the short-bond form of OC–BCl₃, the frequency in the CO region is predicted to be at 2313 cm⁻¹ (shift of +73) and was not observed during experiments. As for the long-bond form, predictions have a vibration at 2248 cm⁻¹ (+7). A peak at 2145 cm⁻¹ (+4) was assigned to the long-bond form. With HX being a problem in experiments, especially because it forms a complex with CO with peaks that register in complex experiments, we also predicted the shift for OC–HCl, which is 2261 cm⁻¹ (+20) and experimentally was assigned to a peak at 2154 cm⁻¹ (+13). Table 2 also includes product band assignments and peak shifts for argon experiments. Peaks were assigned in a similar fashion as described with the neon experiments.

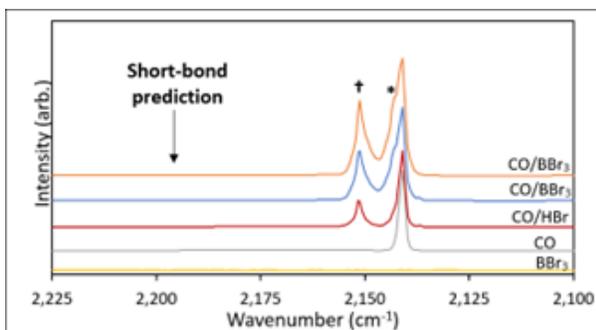


Figure 9: IR spectra of OC–BBr₃ in solid Ne. The assigned complex peak is marked with an asterisk (*) and the impurity peak is marked with a dagger (†). The place where the short-bond form is expected is marked.

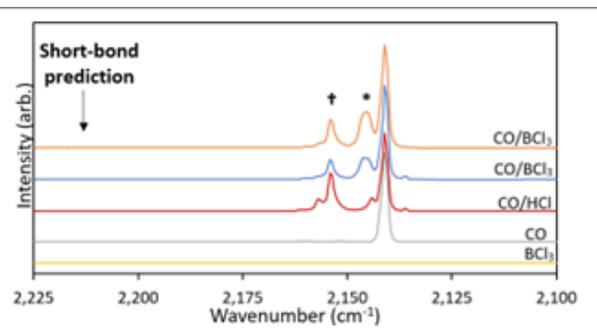


Figure 8: IR spectrum of OC–BCl₃ in solid Ne. The assigned complex peak is marked with an asterisk (*) and the impurity peak is marked with a dagger (†). The place where the short-bond form is expected is marked.

	Frequency (cm ⁻¹)			Shifts (cm ⁻¹)		
	Observation ^a (Ne)	Observation ^a (Ar)	Calculation	Observation ^a (Ne)	Observation ^a (Ar)	Calculation
OC–BCl ₃ (long) ^b	2145	2143	2248	+4	+6	+7
OC–BCl ₃ (short) ^c	-	-	2313	-	-	+73
OC–HCl	2154	2153	2261	+13	+16	+20

Table 2: Observed and calculated vibrations frequencies of OC–BCl₃

Table 3 shows all peak assignments and shifts for the OC–BBr₃ complex. For OC–BBr₃, two product peaks were also seen. Like OC–BCl₃, the short-bond form of the BBr₃ complex, calculated at 2230 cm⁻¹ (+56), was not observed. The long bond-form was predicted at 2246 cm⁻¹ (+5) and was observed at 2143 cm⁻¹ (+2), a small shoulder off of the free CO peak. The OC–HBr complex is calculated to have a peak in the CO region at 2244 cm⁻¹ (+3). The peak at 2152 cm⁻¹ (+10) we assign to the HBr impurity.

	Frequency (cm ⁻¹)		Shifts (cm ⁻¹)	
	Observation ^a (Ne)	Calculation	Observation ^a (Ne)	Calculation
OC–BBr ₃ (long) ^b	2143	2246	+2	+5
OC–BBr ₃ (short) ^c	-	2230	-	+56
OC–HBr	2151	2244	+10	+3

Table 3: Observed and calculated vibrational frequencies of OC–BBr₃

a) Margin of error: 1 cm⁻¹. b) Bond length: 3.483 Å. c) Bond length: 1.604 Å.

As was stated before, we moved to CO after signals from the nitriles proved to be confusing with various overlap, and this move proved to be a good decision as we were able to see the smaller shifts of the long-bond form of each complex. However, the short-bond form, which has much larger shifts, was not seen in any experiments.

Conclusion

We have conducted a computational and experimental study of a series of OC–BX₃ complexes, examining their structural and energetic properties. The OC–BF₃ has a singular structure with a long and weak B–C bond. An interesting observation is that while the BF₃ complex has a potential energy curve reminiscent of a system prone to slight condensed-phase medium effects, and comparisons between previous experimental data and our predicted frequencies show that in an argon matrix there is difference between observed and calculated frequencies indicative of a slight compression of the B–C bond. Computationally, OC–BCl₃ is predicted to have two structures, a short- and long-bond form with the lowest energy form that of the long-bond structure; however, IR experiments were only able to assign peaks to the long-bond form. Much like the BCl₃ complex, OC–BBr₃ shows the potential for two distinct structures computationally, but the short-bond form is lower in energy. Also, IR spectra show evidence for the long-bond form only. Overall, the trends in the features of the B–C potential energy curves can be rationalized by properties of the BX₃ acceptors and seem to illustrate a delicate interplay between attractive and repulsive forces along the B–C coordinate.

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How Adverse Childhood Experiences Affect the Mental Health and Academic Performance of College Students

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Abstract

Questions about the mental and physical health of individuals who have experienced trauma is a topic that continues to gain attention. Some areas of focus include the extent of a person's trauma, the effect of trauma on future mental and physical health, the impact trauma has on an individual's interactions with others, and the ability to form new relationships. Studies show that higher Adverse Childhood Experience (ACE) scores are directly associated with negative mental health outcomes (Chamberlain, 2020). In this study, I analyzed the content of 105 survey responses from college students to explore the self-reported impact of ACE scores on academics and relationships.

Literature Review

Significant research has been done using the Adverse Childhood Experiences (ACEs) survey as a starting point for defining what constitutes a traumatic event in a child's life. In a study published in the American Journal of Preventive Medicine, 10 different ACEs that an individual could experience between the ages of 0 – 18 years of age were studied (Felitti, Anda, Nordenberg, et al, 1998). These 10 childhood traumas include physical abuse, emotional abuse, sexual abuse; physical neglect, emotional neglect; mental illness of a family member, the incarceration of a relative, violent treatment of the individual's mother, substance abuse within the home, or divorce. For each of these events that a child experienced, even if only once between the ages of 0-18 years of age, the child's ACE score increases. For example, if an individual experienced physical abuse one time between the ages of 0-18 years of age, the individual would have an ACE score of one. If an individual experienced physical abuse multiple times between 0-18 years of age, but did not experience any other ACEs, the individual would still have an ACE score of one. The following review of the literature offers some background on ACEs to enable readers to better understand the research.

In a cross-sectional study by Chamberlain (2020), college students' adverse childhood experiences, psychological capital, overall well-being, and mental health were examined. Participant's completed a questionnaire with each question relating to a different ACE. Researchers utilized the Keyes Mental Health Continuum-Short Form (MHC-SF) to measure the participants' wellbeing. Participants rated their emotional, social, psychological, and mental health ranging from languishing to flourishing. Participants also completed the PsyCap questionnaire which measured participants' hope, efficacy, resilience, and optimism. The

objectives of the study were to evaluate the relationships between the student's ACE score, mental and physical health, and overall wellbeing. Descriptive statistics, correlations, and one-way ANOVAs were used to find out the statistical significance within these relationships. The research found that students who scored an ACE of 0-3 had a negatively correlated score with the MHC-SF score; however, there was a positive correlation between the students PsyCap score and total MHC-SF score. The mean PsyCap score ($M=104.45$, $SD=19.95$) for the students with an ACE score of 0-3 was higher than the mean PsyCap score ($M=101.80$, $SD=15.28$) for students with an ACE score between 4-10. According to these means, the higher the person's ACE score, the lower their PsyCap score, showing that students who have had more adverse childhood experiences are more likely to possess less hope, efficacy, resilience, and optimism at the time of the study. The effect size as stated by Cohen's d is a small effect size ($d=0.18$). The mean MHC-SF score ($M=46.49$, $SD=14.16$) for the group who scored 0-3 on the ACE was higher than the mean MHC-SF ($M=38.05$, $SD=15.04$) for the group who scored 4-10 on the ACE. The effect size as stated by Cohen's d is a medium effect size ($d=0.58$). These results suggest that a higher ACE score is directly associated with negative physical and mental health outcomes. Students who have had more ACEs are predicted to experience more mental health issues or report having a lower overall wellbeing. Based on these findings, early intervention for children who experience any of the ACEs should be considered.

Another experiment conducted with a focus on college students found similar results. However, instead of comparing ACEs to mental health and wellbeing, the researchers compared ACE scores to academic success (Karatekin, 2020). This study was a quantitative correlational survey design. It consisted of four separate adapted surveys administered over three separate terms. The surveys had a response rate of 1,197 (N) college students² total. The purpose of the first part of the study was to see if a relationship existed between ACE scores and students who reported first-generation status as a college student. The second part of the study was used to determine prediction values for the grade point average (GPA) of student participants. To determine these predictions, researchers used demographic data and ACE scores. After collecting their data, they found that 59% of students reported experiencing at least one ACE, 38% of students reported experiencing two or more ACEs, and 22% of students had an ACE score of 4 or more. Higher ACE scores existed among those students identifying as first-generation students compared to the multigenerational students in the study. Researchers also predicted lower GPA for first-generation, male, African American, or multiple race-ethnicity students who had ACE scores of four or more. This data suggests that college students who have higher ACE scores are more likely to identify as first-generation college students and are predicted to have lower GPAs. Based on this prediction, college students who report a higher ACE score are at an increased risk of poorer academic performance as measured by GPA. This likelihood is increased if they are first-generation students, or people of color. This evidence reinforces that early intervention should be in place for children who experience ACEs.

Studies conducted involving the general population were explored too. One study investigated the relationship between childhood trauma and the quality of social networks and health outcomes later in adulthood (Schnieder, et al. 2017) Researchers used a convenience sample of 254 adults seen at 10 primary care facilities in the state of Texas. Participants were surveyed to determine their ACE score with additional questions that focused on whether they had any stressful or supportive social relationships, medical conditions, anxiety, depression, and their

health-related to the quality of life. After administering these tests participants were placed into four different ACE classes: minimal childhood abuse (56%), physical/verbal abuse of both child and mother with household alcohol abuse (13%), verbal and physical abuse of a child with household mental illness (12%), and verbal abuse only (19%). Researchers found statistically significant differences between the four-classes when examining mental health outcomes in adulthood. The participants assigned to groups that had compromised mental health in adulthood were more likely to have been children who were physically and verbally abused, but this was found to be even more prominent in participants who witnessed physical abuse of their mother. Researchers noted that participants who reported having supportive social networks as adults, suffered lower odds of reporting poor mental health regardless of ACE experiences. On the other hand, individuals who had increased stressful social relationships in adulthood ended up having more adverse mental health outcomes. These findings show that participants who fit into one of the ACE categories given in this specific study, later in life would have worse mental health outcomes in their adulthood. If provided early intervention strategies around social relationship skill building, the children who experienced the ACEs explored in this study could potentially have had improved mental health outcomes later in life.

The next study conducted on the general population that was reviewed focused on the relationship between an expanded ACE score and adult mental health outcomes (Merrick, 2017). Each ACE score was examined separately to determine the contribution of each ACE on the participant's mental health. The study consisted of 7,465 adult participants in southern California. Researchers created Dichotomous variables that corresponded with each of the 10 ACE categories, plus an additional category unique to this study: spanking. Participants stated how many of these they experienced during childhood and that was their reported ACE score. Researchers used multiple logistic regression modeling to examine the relationship between each of the ACEs and adult mental and behavioral health outcomes. Results showed that higher scores were more likely to lead to moderate to heavy drinking, drug use, and suicide attempts by participants in adulthood. In some adjusted models, being spanked as a child, which was the 11th ACE in this study, was significantly associated with all self-reported mental health outcomes showing that participants who experienced ACEs were more likely to struggle with alcoholism, drug use, and suicide attempts. This study strengthens the argument that early intervention is needed for children who have had adverse childhood experiences.

Loxton (2018) explored the topic of medical care and its relationship to ACEs. Researchers followed a cohort of 14,247 (n) adults for 20 years to examine the number of money participants spent on medical care and analyzed this in relation to the participant's ACE score. Researchers found that overall, 41% of the women in this cohort stated they had at least one ACE. The most commonly reported ACE was having had a household member with a mental illness (16%), with the second most common reported ACE being psychological abuse (17%). The study showed that women who reported having had more ACEs had higher healthcare costs indicating that people with higher ACE scores will likely spend more over a lifetime on medical care than people who have experienced fewer ACEs, once again reinforcing the idea that these ACEs have a negative impact on adults.

In a study of 404 college students explored how ACEs related to race/ethnicity impacted academic achievement (Watt, et al 2021). Participants completed surveys and self-reported their

ACE scores, demographics, and GPA. Results found that students of color reported higher ACE scores and lower GPAs than white students. The study also revealed that an ACE score of four or higher was associated with lower GPAs, but only for students of color, not for white students. This research showed that there are race/ethnic differences in the impact of ACEs on post-secondary academic achievement.

A study was done to determine the association of ACEs to protective, familial, and community factors in relation to school performance and attitudes of children ages 6 to 17 (Robles, 2019). The researchers did a cross-sectional analysis of the 2011-2012 National Survey of Children's Health. ACE scores were categorized as 0, 1, 2, 3, and 4 or more. Children's protective factors were defined as: safe neighborhood, supportive neighbors, four neighborhood amenities, well-kept neighborhoods, no household smoking, and more than five family meals per week. Results found that each negative school outcome was associated with higher ACE scores and lower protective factor scores. The study showed that the strongest protective factor for children is the ability to talk with caregivers about things that matter and share their ideas.

All the studies reviewed are similar in the sense that they all involved adults of the general population or college students that continued to suffer from negative effects of childhood trauma. Some of the effects included poor physical and mental health, a higher risk of alcoholism, drug use, or suicidality. Each study reinforced the devastating impact that Adverse Childhood Experiences can have on a person in their adult life.

Introduction

For this research project, I explored how ACEs affect the mental health of college students, the residual impact their mental health has on their academic performance. This is important because with increased understanding of the effects ACEs have on college students there is the potential to increase or develop resources that will better assist students with increased ACE scores to be successful. The hypothesis we had was that an increased ACE score would have a significant impact on academic performance, measured through self-reported interpretation of success, and on relationships, whether it be with friends, classmates, or professors.

Methods

Participants

The survey was sent out to students in Social Work 100 and Psychology 100 classes. The survey was also published on SONA, which is an online survey system that records research participation credit. Students in a psychology classes could take online surveys, and receive either credit or extra credit in that class through the SONA application. Participation in this research was voluntary and a total of 105 participants completed the survey questions.

1.2 Materials

A letter of consent was included at the beginning of the survey outlining that by proceeding with the survey, a prospective participant was giving their permission for their information to

be collected. The survey consisted of 19 questions. Minimal demographic information, such as age and number of semesters enrolled in college were collected. Participants were asked specific questions used to determine their self-reported ACE scores, with follow-up questions that asked participants to rate aspects of their mental health on a Likert scale. The survey also contained three qualitative questions asking participants to describe ways they had seen their mental health impact their college career and relationships, what campus opportunities they have participated in, and what campus opportunities should be added to assist with academic success. The end of the survey contained a message thanking participants for their participation and provided phone numbers for on-campus counseling services, the mental health and AODA crisis line, and the crisis text line.

Results

2.1 Impact on Academics

Question 14 was a qualitative question asking participants to explain how their mental health has impacted their academic performance throughout college, and whether the impact was positive or negative. Participants' self-reported responses were coded in a hierarchical frame by single words based on external and internal factors; with internal factors having negative, positive, and other categories. For external factors the words pandemic (4) and online classes (4) appeared to affect participant's mental health, in turn impacting academic performance. Other mentions included not going to classes (2) and not getting assignments done on time (2). When examining reported internal negative factors, words that appeared most often were stress (17), nervous/anxious (18), unmotivated (19), poor grades due to mental health (13), losing focus (10), and depression (8). Internal factors that were categorized as positive factors included the most often mentioned words or phrases of doing well in classes (4), anxiety helping with classes (3), and being motivated. The last category for internal factors was other, and participants mentioned quality of work (1) and completing assignments (1). Participants utilized 32 unique words coded in the internal positive, or internal other category, and utilized 148 different words to describe the negative internal factors.

2.2 Impact on Relationships

Question 15 on the survey asked participants to record how their mental health had affected their relationships with professors, peers, friends, etc. The open ended responses were coded in a hierarchical coding frame, with there being external factors as one category, and internal factors as the other category. These were then subcategorized as negative, positive, and other. External factors mentioned by participants included the ability to be open about mental health with friends (8), professors not understanding (3), and having a good friend groups (3), with "good" being self-defined by the participants. For the internal negative factors, participants mentioned self-isolation (26), detached from others (12), anxious (12), and stressed (7) most frequently. In the internal other category, participants noted having a hard time making friends (9), being a "burden" to professors and classmates (5), and inability to reach out to teachers or classmates (4). Participants utilized 37 unique words coded in the internal positive, or internal other category, and utilized 88 different words that were coded as negative internal factors.

2.3 Campus Opportunities

Participants were asked in question 17 to state what campus opportunities they have participated in. They were provided with examples such as counseling services, academic success programs (McNair, Blugold Beginnings, Student Support Services,) faculty student research, and first year experiences, etc. . Counseling services (19) was mentioned the most as being used, with the writing center (12) being used the second most, and participants also reported being involved in research (14), intramurals, (9) and tutoring (9). The question 18 on the survey asked participants to state any additional activities that could be added to assist academic success. Mental health days (2) was mentioned, as was more availability in counseling services (4), and the availability of emotional support animals (1).

2.4 Relation of ACE Score to Diagnosis

Participants were asked to self-report whether or not they had a diagnoses of anxiety, depression, or both, and if so, at what age this had occurred. Participants also reported their ACE score through a series of questions on the survey. The average age for an anxiety diagnosis was 13, and the average age for depression diagnosis was 14. Survey participants who reported no depression or anxiety reported an average ACE score of 2.75. Participants reporting diagnosis of both depression and anxiety reported an average ACE score of 3.92. While those reporting only anxiety reported an average ACE score of 5.6, and participants reporting only depression reported an average ACE score of 6.

Diagnosis	Average ACE Score
No Depression or Anxiety	2.75
Both Depression and Anxiety	3.92
Only Anxiety	5.6
Only Depression	6

Figure 1. *Average ACE score with Diagnoses.*

Discussion

3.1 Introduction

Higher ACE scores have been directly associated with negative mental health outcomes, and a lower overall well being (Chamberlain, 2020). For this reason, it could be inferred that higher ACE scores will likely impact college students' academics, and their relationships. Through our research, we found that students' perception is that feeling unmotivated, nervous/anxious,

and stressed, which participants attributed to their mental health, has affected their academics. . Students also reported feeling that behaviors such as self-isolation, being detached from others, and feeling like a burden, which they attributed to their mental health affects their relationships with professors. In contrast, participants also reported that being open about mental health was something that was positive, and was perceived to help relationships.

3.2 Limitations

One limitation to this study was that it was not intended to be representative of the entire world, but more specifically for college students in the United States. Our sample size was relatively small (105) compared to the number of students enrolled at the University of Wisconsin-Eau Claire (9,803) campus. All of the questions were self-report, which is reliant on participant's to answer truthfully and accurately. While many different classes were invited to partake in the survey, it is assumed that the survey likely was completed by a majority of people interested in human sciences. The results may present very differently if the sample was larger and more varied with regard to discipline. The study took place during the pandemic, which will create additional challenges and will be discussed independently.

3.2 COVID-19

This research study was designed and conducted during the 2020-2021 academic year. During that period there was a global pandemic occurring that dramatically impacted the organization of college life; from classroom structure to socialization and access to in-person resources. Each of these factors independently could have affected the data, however it is likely that with all of these elements occurring simultaneously, that the pandemic changed participants' mental health, affecting the data collected.

3.4 Strengths and Weaknesses

This research has numerous strengths and weaknesses. By including the survey on SONA, and with some professors offering extra credit for participation in research studies it is likely we increased our participation. By having the survey available to Psychology 100 students it increased the likelihood of a varied participation of majors, since this is a course taken across disciplines. Weaknesses include the number of questions. The survey contained 19 questions, which could deter people from completing the survey. The use of SONA could also be a weakness because it is not a resource used by all disciplines and may be more specific to psychology students. The survey also contained seven questions that the data was not used in the final results. Elimination of these questions would have reduced the length of the survey, as well as the potential risk of emotional upset to participants. The survey also asked participants questions about their

Conclusion

Higher ACE scores have been noted to be directly associated with negative mental health outcomes, and a lower well-being overall (Chamberlain, 2020). When taking this information into account, our data shows that college students experiencing mental health challenges have more self-reported negative experiences, and problems with professors, peers, and friends. This could mean that a college student who presents with a higher ACE score would experience increased challenges during the college career resulting in a negative impact on academics and

relationships.

When looking at the affect that mental health has on student academics, we see from the data that many participants felt their mental health made them unmotivated, anxious, and stressed. With regard to relationships, their mental health made them isolate, feel detached from others, and even feel like a burden to professors and peers. It is important to note that the impacts of isolation and feeling like a burden were reported by participants as affecting relationships. However, it is possible that these mental health responses could transfer to academic impact when connected to academic success (grades) linked to attendance, class participation, or communication to request extensions or accommodations.

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Black Masculinity in the Civil Rights Era: Non-Violent Protest Versus Armed Self- Defense

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The Civil Rights movement of the 1960s was spurred on by greatly impactful events and created lasting changes to the way our nation interacted with Black Americans. As the movement grew, two factions developed, and each of these groups represented a unique set of ideals and methods to achieving true equality for all. One of the key factors in these ideologies is based in the concepts of Black masculinity and how each group viewed the role of Black men in American society. From “respectability politics” and non-violent protest, to armed self-defense and direct action, this presentation will explore the origins of these concepts and demonstrate how they were used. The unique stereotypes and expectations placed on Black men in the United States greatly impacted these different methods, and had lasting influence on the effectiveness of the 1960s movement.

Introduction

The Civil Rights movement of the 1960s was spurred on by many impactful and often terrifying events, and ultimately created lasting changes to the way our government and society interacted with Black Americans. As the movement grew, Black people from a variety of backgrounds began taking leadership positions in social justice organization, and eventually, a split occurred, creating two primary factions of the movement. One of the key factors in the cause of this split was rooted in each groups’ ideas of what Black masculinity represented, and the unique experiences they had with racism and police presence. Jim Crow laws, voter suppression, and lynching, among other things, impacted each group in specific ways, and created a different set of needs for each part of the community, which required contrasting courses of action to address them. The distinct stereotypes and expectations that were placed on Black men in their local communities, and the United States overall, was one of the leading factors in the development of these individual methods. The way each group engaged with the fight for equality greatly impacted the movement as a whole, and while there was some success, the lack of effective police reform and economic empowerment that was needed is attributed to this ideological split.

The History of Anti-Black Stereotypes

American slaveowners required an excuse to uphold their unique brand of race-based slavery. The stereotypes of the mammy, the coon, the sambo, and the jezebel, created to dehumanize Black people and justify their enslavement, carried over into emancipation and the twentieth century. During the 1800s, abolitionists also used these depictions to support their cause, claiming that Black slaves, while human, were incapable on their own. “...Blackness, this sense

of time-space is interrupted by a more weighty and seemingly more truthful...underside – where Black is naturally malignant and therefore worthy of violation; where Black is violated because Black is naturally violent; where Black is naturally less-than-human and starving to death and violated; where Black is naturally dysselected, unsurviving, swallowed up; where Black is same and always and dead and dying; where Black is complex and difficult and too much to bear and violated.” (Abdur-Rahman, p. 3) These stereotypes also blurred gender distinctions under the realm of racial distinction, further dehumanizing those with darker skin.

The viability of Black maleness was minimized during slavery, when children born to a Black slave woman were decreed to hold the same social station as their mothers; children born to two white parents were held to the social station of their fathers. This released white slaveowners who raped their slaves from paternal responsibilities to any mixed-race offspring. It also relieved any white offspring who the slaveowner fathered from social and fiscal competition that the slave woman’s child may have presented. By demasculinizing Black boys and men, white men kept authority over Black bodies even after slavery was abolished. (Staidum, Jr., pp. 18-20)

This parental dynamic gave Black boys a unique relationship with their mothers – their Blackness, which was inherited from her, made them killable and violable, but is simultaneously wrapped in the distinct brand of Black motherhood that could save their lives. According to Aliyyah I. Abdur-Rahman, associate professor at Brown University, “...being Black and Black being come from the mother line. For Black masculinities, the source of ruin [is] perpetual vulnerability and violability.” (p. 40) By connecting Black boys to their heritage exclusively through the mother line, their own femininity was exaggerated, and any masculine traits embodied by a Black male were treated as a threat. “...Black masculine people are imputed with fantastical power and potency (cast as brute, buck, thug, gladiator, sex god/fiend) and simultaneously subjected to spectacular debasement and hurt (made slave, prisoner, lynched body, bullet-ridden corpse) vis-à-vis antiblackness.” (Bost et al., p.4) After emancipation and with the rise of Black Codes and Jim Crow laws, “...the imminently killable non-subject” (Abdur-Rahman, p. 3), the emasculated Black man, was turned into a terrifying and morally bankrupt figure – the Black Male Rapist.

Attacks on Black Bodies

Violence against Black Americans was used to enforce the institution of race-based slavery in the United States. After Emancipation, former slaveowners and others needed an excuse to continue this violent behavior toward their African-American neighbors. This is when many state and local governments began creating the “Black Codes,” which were mandates and laws that criminalized otherwise standard behavior, such as waiting for the bus or getting laid off or fired. These rules were frequently written in the same language as the Slave Codes, and while many were not explicitly directed at Black peoples, their enforcement was almost exclusively based on the alleged offender’s skin color.

Any incident of race-based violence was defended with ideas of racial hierarchy and Social Darwinism. Rooted in Enlightenment concepts that encouraged colonization and purported the “white man’s burden,” these ideas became the basis for medical and scientific experimentation

on Black men and women. The most well-known of these experiments is the syphilis study conducted by the Tuskegee Institute. Lasting for 40 years, the study failed to inform participants of their diagnoses, used experimental treatment without disclosure, and left hundreds of syphilitic Black men untreated for decades, infecting their partners and in some cases, their children. After several news reports on the illegality of the study, it was halted in 1972. It took two years to reach a legal settlement on behalf of the Black families harmed by this study, but a quarter of a century passed before President Bill Clinton publicly apologized to the victims on behalf of the nation.

Another medical intervention enacted against Black bodies was the use of sterilization and eugenics to minimize the Black population in the United States. "The movement, which began in 1904, was a government-sponsored social engineering project which sought to improve the human species by encouraging "fit" people to marry and procreate while sterilizing and prohibiting unions between the "unfit.'" (Coale, p. 1) The scientists behind these projects defended their actions with scientific evidence, arguing "...that non-whites were genetically inferior to whites... The eugenicists' claims were touted by opportunistic politicians..." (Manjoo, p. 4) In one study, Black people housed in public institutions, including poorhouses and women's shelters, were given IQ tests, analyzed for physical markers of alleged inferiority, and were observed for lifestyle abnormalities that were supposed to indicate moral deficiencies. "These facts became the basis of a landmark 1927 Supreme Court decision that allowed states to forcibly sterilize people who carried "hereditary defects." Carrie Buck was forcibly sterilized, and by the mid-1930s, about 20,000 people in the United States met the same fate under similar laws." (Coale, p.1) The story of the Buck family is not unique; many women of color were court-ordered to be sterilized under this precedent.

The most prolific excuse for the deaths of Black men is the threat they pose to white women. The myth of the Black male rapist is a relic of the Jim Crow era, where countless Black men and their defenders were extra-judiciously slaughtered by vigilantes and local police. However, the same fate would not await a white man who laid hands on a Black female: "One of racism's salient historical features has always been the assumption that white men – especially those who wield economic power – possess an incontestable right to access to Black women's bodies." (Davis, p. 175) This played out during slavery as Black women being raped and/or manipulated into bearing bi-racial children, fathered by white men who would never willingly acknowledge the children's parentage. Their white wives would abuse the Black women whom their husbands assaulted. Children born to Black mothers were automatically bestowed with their mother's social status – this was to protect the fathers from financial obligation, as well as to protect white children of shared parentage from competing with their mixed siblings.

After Reconstruction, as Jim Crow laws and Black codes took over towns across the nation, rape or assault accusations by a white woman were enough to get any man hung, but it was only marginalized communities that experienced innumerable counts of vigilante justice. "When Ida B. Wells researched her first pamphlet against lynching, published in 1895 under the title *A Red Record*, she calculated that over ten thousand lynchings had taken place between 1865 and 1895." (Davis, p. 184) After the fact, many of the accusers were caught or admitted to lying. None of the murderers met the same fate as the lynching victims.

Lynching was not reserved for Black men; it was frequently enacted upon white abolitionists and sympathizers with the cause for Black equality. Women were also not immune from the brutality of lynch mobs. In *Rape, Racism, and the Myth of the Black Male Rapist*, Angela Davis recounts Ida B. Wells's recollection of "...the horrible case of the woman in San Antonio, Texas, who had been boxed up in a barrel with nails driven through the sides and rolled down a hill until she was dead." (Davis, p. 191)

The accusation of rape to justify lynching might be attributed to what Susan Brownmiller refers to as "[t]he historic price of woman's protection by man against man was...a crime committed against her body [which] became a crime against the male estate." (Brownmiller, p. 17) Despite Brownmiller's repeated references to "racial differences", which uphold the false narrative of men of color being prone to violence and rape, her position on the frame of femininity within the context of rape provides some insight to the uniqueness of masculinity in marginalized communities.

However, the most visible attacks on Black communities took place in the form of separating Black neighborhoods and then overpolicing those areas. This resulted in unprecedented miscarriages of justice, and skewed crime data that was then used to excuse violent treatment of Black suspects and the Black imprisoned. To police and government institutions, a Black person posed a threat, regardless of gender: "...Black genders are merely the exploited outcomes of distinct modes of racial terror." (Abdur-Rahman, on the murder of Korryn Gaines in 2016 by the hands of Baltimore police SWAT, p. 3) Using "...the notion of ineradicable race differences" to create legislation resulted in unequal application of laws, especially those laws which pertained to violence. (Handlin, p. 211).

Legislation and Actions Against Black Progress

Anti-Black legislation, mandates, and court rulings created a deep web of generational oppression and dehumanization of Black people across the United States. The nuances of Reconstruction, Jim Crow, and segregation and their long-term effects on Black communities goes all the way back to Emancipation. President Lincoln's Proclamation was made in January of 1863; however, on June 19, 1865, two and a half years later, Texan slaveholders had still refused to release their slaves. The federal government, under the urging of Black soldiers who had risked their lives to fight for the Union, sent a military operation to Galveston, Texas, ordering slaveholders to grant all slaves full freedom, including paid wages and fair labor.

This action was just the beginning of Reconstruction, which took place from 1866-1877. During this time, the U.S. Congress passed and ratified the 13th, 14th, and 15th Amendments, along with the Civil Rights Act of 1866. These ensured citizenship, voters' rights, land ownership rights, fair employment, and use of public spaces for Black men, and in part for Black women ("The African-American Odyssey"). Federal troop presence in the Southern states protected Black communities from white supremacist retaliation, and the Enforcement Acts of 1870 and 1871 offered protection to Black families. In 1877, the presidential election caused intense conflict between the North and the South once again. In a last ditch effort to preserve a Republican

administration, Northern representatives took the bait when Southern Democrats said they wouldn't contest Rutherford B. Hayes' presidency – if all federal troops were removed from the Southern states. Without viable enforcement, Southern white supremacists monopolized control over community economies, police forces, and government offices (“The Civil War and Reconstruction”).

Debt peonage laws allowed Black people to be forced into indentured servitude for even the smallest debts. Sharecropping demanded that Black farmers pay for the use of land, equipment, and kept Black families in isolated Southern communities. These systems created traps of generational poverty, making it harder and harder for Black families to gain upward mobility, or even to relocate in search of better opportunity (“The Civil Rights Act of 1964”).

The formation of the Ku Klux Klan in 1865 sparked terror in Black communities and their white allies across the nation. Despite the idea that the KKK was only prevalent in the South, there were chapters throughout the North and West, as well, targeting various communities who the Klan decided didn't fit their idea of the “superior white.” Other white-supremacist organizations also formed, and these groups infiltrated police and government organizations, giving them an extra layer of defense against prosecution as they threatened, assaulted, and murdered thousands of people. Members were rarely prosecuted, and almost never convicted. Many of these groups, and their founders' descendants, still have a presence in the United States today.

Meanwhile, Washington, D.C. offered no respite. The Supreme Court ruled in favor of segregation in 1896 in the case of *Plessy V. Ferguson*, making room for the Black Codes of the Jim Crow era to go into full effect. These mandates included vaguely worded “vagrancy laws,” which allowed the arrest of a Black person for nearly anything, even just sitting at a bus stop. The accused were typically put to work in unpaid labor houses. All Southern public facilities were mandated to be segregated, and in 1913, President Woodrow Wilson extended this mandate to the military and federal offices. Legal segregation was not overturned until 1954, in the landmark Supreme Court case of *Brown V. Board of Education*, when it was determined that segregated facilities could not be “separate but equal.” (“The Civil War and Reconstruction”)

As soon as federal troops withdrew from the South, anti-Black measures were quickly enacted. For example, the previously integrated state university of South Carolina promptly shut down, reopening as an all-white university three years later. Harvard's first Black graduate was a professor there; his job vanished, along with integration. In Louisiana, voting restrictions such as literacy tests and limited poll access, caused the Black male voter registration to drop nearly 99% in less than five years. (Gates, Jr.)

Redlining was the act of re-zoning residential areas to legally segregate Black and other marginalized communities from the rest of the population. It also limited BIPOC access to good-paying jobs, adequate housing, and even created barriers to practicing the right to vote. This practice, and the rise of racial ideologies helped create the “Negro Ghetto” in cities across the nation, including the very diverse mini-melting pot of Chicago, Illinois. In the late 1860s, there were almost no all-Black blocks and most neighborhoods were well-integrated; “[b]y 1915... the physical ghetto had taken shape; a large, almost all-Negro enclave on the South Side, with

a similar offshoot on the West Side, housed most of Chicago's Negroes." (Spear, p. 219) This played out in similar ways in nearly every state, with Black ghettos emerging in New York, Los Angeles, Detroit, Philadelphia, and many other metropolitan areas during the same period. The effects of these actions are still seen today in urban neighborhoods that are predominantly communities of color, are largely underfunded and have limited access to resources available in other, white-dominated neighborhoods. "The housing problem for Negroes was not restricted to the poor; even the affluent were blocked in their quest for a decent place to live." (Spear, p. 223) This created a distinct racial wealth gap between communities of color and white neighborhoods. Upward mobility was difficult for most, but extremely restricted resources and the need to commute to decent paying jobs, often requiring upwards of an hour on freeways and toll roads that poor communities couldn't access on public transportation, made "moving on up" a near impossibility for members of these redlined neighborhoods. Redlining Black communities also made overpolicing and unequal enforcement of law easier to conduct, and much harder to prove to those outside the community.

The legislation passed during this period across the United States demonstrates the ideology that, no matter how upstanding and revered a Black man is, the white man or woman has the power to reduce them to another violent, animalistic face in a sea of Black men. According to post-Reconstruction lawmakers, "...the Negro was inherently inferior, did not need or deserve, could not use or be trusted with, the rights of humans." (Handlin, p. 210)

Mass Incarceration

The Slave-Codes-turned-Black-Codes brought about the chain gangs and ghettoization of early-twentieth century America. Black fathers were ripped away from their families and sentenced to unpaid labor, and redlining and gerrymandering sliced up cities into segregated neighborhoods and funneled taxpayer dollars into stable white-dominated neighborhoods. Marginalized communities, who desperately needed infrastructure invests in education, healthcare, jobs, housing, and roads, had more and more of their tax funding reallocated to already-secure areas. Lawmakers who allowed these policies across the nation believed that "...[Black] racial inferiority justified a position of permanent subordination." (Handlin, p. 211) This is an area in which meaningful progress is still lacking, as thousands of Black men and women are serving long-term prison sentences for misdemeanors and other non-violent crimes today.

Beginnings of the Civil Rights Movement

The beginnings of the Civil Rights movement can be traced back to the 1790s, when the United States was a brand new nation, and slavery was legal in most states. There are several examples of slaves who petitioned for freedom, or free Blacks who petitioned for equal rights. ("Free Blacks Petition", pp.163-164) This momentum was continued after emancipation, through the Jim Crow era. Lynchings and rising police brutality in marginalized neighborhoods led Black leaders to organize and put an end to racial violence and inequity.

The Student Nonviolent Coordinating Committee was founded in April of 1960 as the result of a meeting called by Ella Baker, who was concerned that Dr. King and the Southern Christian

Leadership Conference (SCLC) were not relatable or appealing to young adults who were looking for ways to make more meaningful changes, more quickly. SNCC participated and helped organize the Freedom Rides and voter registration drives. The passion of youth soon overtook the organization; three members were killed by the Ku Klux Klan in 1964, and in 1966, Stokely Carmichael was elected to head the organization. The creator behind the Black Power movement, Carmichael advocated for self-defense in the form of violent retaliation – giving young Black members the support they needed to finally fight back. This resulted in escalated clashes with white conservatives and police, and in 1967, the organization was disbanded after Carmichael's successor, H. Rap Brown, was arrested for incitement to riot. ("SNCC")

In 1909, Oswald Garrison Villard issued forth a call to action for the protection and advancement of the Black community. As lynchings became more common and Jim Crow laws made it more difficult for Black people to exercise their civil rights, a group of Black leaders came together to form the National Negro Committee; on the NNC's one-year anniversary, May 30, 1910, the name was changed to the National Association for the Advancement of Colored People. The organization was created to be interracial, and conducted legal work against racial injustice and other inequalities. The NAACP's special publication, *The Crisis*, was edited by W.E.B. Du Bois, and the leadership was full of recognizable names. However, some whites and conservatives felt that the NAACP was "radical," and other Black advancement organizations refused to support the association. Despite this adversity, the NAACP is still an active and influential organization today. (Grant et al., 210-214)

Civil rights organizations across the nation began voter registration drives, encouraging Black citizens to engage in democracy and politics. The urban drives were largely successful, but many rural Black communities did not have access to reliable transportation and were terrorized by the white Southerners in their area. This made it both difficult and unsafe to travel to a courthouse to register, especially with police officers ignoring or even participating in the harassment and assault. Armed guards and armed self-defense advocates protected organizations and members from white intimidators and violence. (Grant et al., 258-259)

These organizations and their members' participation in political activism led to the passage of the Civil Rights Acts of 1960 and 1963. "Lack of urgency in Washington" was cited as an issue by Black leaders, as endless meetings with government officials led to watered-down bills with limited enforceability; this was a slap in the face to Black leaders who felt that asking for equal treatment wasn't a big ask. This sentiment "Either party is welcome to claim credit for enactment of the wretched remnant of what was not very much at the outset." (Grant et al., 368) Discontent with passive stance of President John F. Kennedy (most far-reaching legislation of its kind, but failed to include "a fair employment practices provision" (Grant et al., 368)) Government's goal in the 1960s, it seems, was to appease the masses, not to pursue justice and democracy for all.

The Voting Rights Act of 1965 was passed with the goal of making the Fifteenth Amendment enforceable. This act banned literacy tests, poll taxes, and intimidation tactics that were frequently used to keep Black citizens from voting. Some organizations advocated for special municipal elections after this bill was signed into law, to ensure Black voters were properly represented after the upcoming election. While this wasn't included in the bill, a suit was brought

in Sunflower County, Mississippi proclaiming the regular local election invalid. The suit was won, and despite white intimidation, Black voters turned out in droves, and they lost the special election by only 30 votes. (Grant et al., 369) Several states challenged the act in court, but several Supreme Court decisions, in cases such as *South Carolina V. Katzenbach* and *Allen v. State Board of Elections*, upheld the constitutionality of the act, and by the end of 1965, 250,000 Black voters were registered to vote for the first time.

In 1964, white middle-class students traveled to Mississippi to help with voter registration and to teach in Freedom Schools. When two of these young workers disappeared with one of their Black peers, federal action was finally taken. The Mississippi and Louisiana rivers were dragged in search of the young men; countless dismembered Black bodies were pulled up from the bottom. Finally, the nation could no longer deny the terror that Black citizens had faced, and a report on racial violence was done by the Southern Regional Council. However, despite massive efforts by Black-led political organizations to achieve equal representation for Mississippi Blacks, they were not exactly welcomed by mainstream Democrats. Voter suppression and economic oppression based on race were never efficiently eliminated.

Conflicts in the Black Experience

Economic competition and biases based on the previously discussed stereotypes influenced how white people categorized Black members of their community. Anti-Black and anti-immigrant propaganda created and strengthened racial chauvinism, built on the ideal of racial purity. The “white Christian savior” trope offered a mask to hide covert racism, and when unsolicited and harmful advice was rejected by the Black community, the “white saviors” used this as proof of racial inferiority. White concepts of what a “respectable citizen” looked like and behaved shaped their interactions with Black people, and that experience could change drastically based on the context, environment, and subject of the encounter. These variables affected the way different types of communities thought they could achieve racial equality and civil rights.

In 1899, Rudyard Kipling wrote and published the poem “The White Man’s Burden,” which expressed the author’s opinion that people of color were “half devil and half child” and that it was the white man’s obligation to colonize, capture, and convert anyone who was not born with the blessing of whiteness. (Kipling) This rhetoric shaped many Progressive Era policies that directly harmed the Black community. These policies included eugenics programs, ghettoization, and pseudoscience-based propaganda campaigns that instilled a deep-seated contempt for anyone with darker skin. These efforts also targeted ethnic whites in the beginning, and were marketed as if to save marginalized people from their own self-destructive behavior, although there was no evidence for such a proclivity. This concept is the basis for the white savior complex, in which white individuals or institutions cause harm to the Black community by implementing or suggesting ideas based on bias instead of on the Black experience.

Respectability is a concept based on the idea that successful individuals looked, spoke, and behaved a certain way, and anyone who did not follow these recommendations were a “hopeless case,” destined to live in squalor and disgrace. “The politics of respectability entailed “reform of individual behavior as a goal in itself and as a strategy for reform.” Respectability was

part of “uplift politics,” and had two audiences: African Americans, who were encouraged to be respectable, and white people, who needed to be shown that African Americans could be respectable.” (Harris, p. 213) These ideals were adopted and adapted by some of the Black community, regarded as the only way to claim “equal status and citizenship during the Progressive era.” (Harris, p. 219)

However, implicit and overt biases proved itself much harder to overcome than expected. “The informal economy, the diverse ideologies it fostered, and the leaders it spawned lessened the influence of bourgeois respectability within the African American community. At the same time, it was becoming clear that the tactic of bourgeois respectability was not effective in changing white people’s racial attitudes.” (Harris, 215) Despite massive efforts to police their own behavior and embody white ideals of propriety, Black citizens couldn’t avoid racist treatment and policy.

Some, mostly middle-class educated Black individuals, blamed the poor, urban Black neighborhoods for these failures: “...a small, compact, but rapidly growing community divided into three broad social groups. The “respectables” – churchgoing, poor or moderately prosperous, and often unrestrained in their worship – were looked down upon somewhat by the “refined” people, who because of their education and breeding, could not sanction the less decorous behavior of their racial brothers. Both of these groups were censorious of the “riffraff,” the “sinners” – unchurched and undisciplined.” (Spear, 217) As the Black community turned inward and began to divide, the civil rights movement split between the “refined/respectables” and the “riffraff,” each group taking its own path and making its own demands. This split caused tension between the movement and the government, and eventually, several organizations from both camps disbanded, causing the overall movement to lose traction, both in media and in government.

Relevance to Today

The effects of systemic racism and white patriarchal ideals continue to cause issues for the Black community and other marginalized groups. “Today’s continuing inequalities in education, housing, employment, wealth, and representation in leadership positions are rooted in our country’s shameful history of slavery and systemic racism.” (United Conference of Catholic Bishops, 1) In the United States, median wealth for white households is ten times greater than for Black households, and eight times greater than for Hispanic households. “Unemployment rates for African Americans, Latinos, and Native Americans are considerably higher than the national average. Growing income inequality increasingly affects marginalized groups. Minority homeownership rates lag behind their white counterparts, and yet research shows that minorities face extra hurdles in getting approved for mortgages. African Americans, Latinos, and Native Americans are disproportionately affected through every stage of the criminal justice system, despite the evidence that different racial and ethnic groups commit crimes at roughly the same rates.” (United Conference of Catholic Bishops, 1)

The solution for correcting these issues is not to assert white goals and experiences onto Black communities, but instead, we need to listen to those most affected by these disparities and apply

resources appropriately to make their goals attainable. The unique situation that Black males face requires a specialized type of policy reform. "...Differences, and those who are "different," must be acknowledged in intellectual work and welcomed in activist circles." Conversations surrounding intersectionality between race, sexuality, and class is key to understanding the issues that face marginalized communities and the individuals within them. (Harris, p. 219) Scholar Joshua Bennett agrees: "My sense is that any such endeavor would necessarily begin with the voices of Black men and boys themselves," and indeed, only Black men and boys can share their experiences with us. (Bennett, 31)

The contemporary Black male experience "...is rooted...in...a systematic, ravenous hunger for Black male flesh, a desire to view, consume, and use Black men and boys as...raw material.... In the contemporary context, this yearning is made legible in the form not only of state-sanctioned ritual killings at the hands of police, security guards, and vigilantes, but in the virtual afterlife of that violence. The footage of Black men killed on camera that circulates for days, weeks, and months on end. Only in death do they reach their full potential. Even in death, they are said to take up too much space. Their corpses crowd the discourse. The gathered masses observe, take their fill, and look away." (Bennett, p. 27) How can they be heard when, in life they are muted, in death they are disparaged, and in memory they are pushed aside?

Today, the approach we take to social justice efforts should bear the mark of the history of the movement, and one of the most effective methods that has proven effective is to dismantle the systemic barriers that are built into existing and pending policy and legislation. "Racism can only end if we contend with the policies and institutional barriers that perpetuate and preserve the inequality—economic and social—that we still see all around us. – U.S. bishops, Open Wide Our Hearts." (United Conference of Catholic Bishops, p. 2) Another lesson we should carry forward is the fact that turning a blind eye to racist behavior in any context only perpetuates the damage caused to Black communities across the nation. "Openly rejecting stereotyped joking [is] an anti-racist way that reflects a continuing use of the equality-and-justice framing of society." ("Two-faced Racism", p. 15) Finally, admitting our own inherent biases and actively working to correct them is key to enacting lasting and meaningful change. For instance, claiming to be color-blind, in a way to deflect evidence of one's own racist behaviors and biases, ignores the impacts that institutionalized racism has had on communities of color, and only works to uphold those same institutions. If one is called out on their racist rhetoric or behavior, even if one's intention was not to be disparaging, one must apologize for the wrong done, admit their personal gap in knowledge regarding the harmed person's experience, and work to correct this even when not in the presence of a marginalized person.

Conclusion

While many of the initial goals of Civil Rights advocacy were reached, many Blacks today are still trapped in the oppressive cycle of institutional racism. Because the experiences of urban, low-income Blacks were so different from the experiences of middle-class Blacks, more impactful actions were difficult to maneuver. The same stereotypes that excused the enslavement and abuse of Africans and Black Americans for 400 years still exist today. Countless organizations, including the ACLU and Black Lives Matter, continue to push for equity. As in the

1960s, college students continue to be some of the primary participants in the revived fight for equality.

The effects of slavery, racism, and the stereotypes surrounding Black maleness are still prevalent in the disproportionately high numbers of Black men who have been imprisoned, Black families trapped in generational poverty, and Black peoples who have been killed by police and white supremacists, who receive little to no repercussions in most cases. Racist rhetoric has been deeply ingrained into the fabric of American society, and the 1960s movement only laid the foundation for all the changes necessary to undo those stitches. Today, Black Lives Matter has replaced SNCC and the Black Panthers, and continued activism, even in the face of intense adversity, is building on the groundwork of the past to teach empathy and correct implicit biases. By understanding Black masculinity and the politics of the Black community, we can create a better understanding of our nation's past, and work together toward a more equitable and just future.

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Predicting Burnout at Work from Personality Traits and Work Factors

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Abstract

Burnout is defined as a chronic, negative, work-related psychological state; it is predictive of job dissatisfaction, low levels of productivity, and job turnover. Burnout is particularly common for individuals working in people-oriented professions, including healthcare and education. Previous research has established links between burnout and a variety of work factors, such as a lack of control and autonomy at work. Research has also established links between personality traits, such as negative emotionality, and susceptibility to burnout. In the current study, we measured burnout, fourteen specific work factors, and five broad personality traits for two samples of workers: (1) faculty and instructional staff at the University of Wisconsin-Eau Claire, and (2) Mayo Clinic physicians and nurses. Results indicate nearly half of Mayo respondents, and a third of UWEC respondents, scored high in burnout. In both samples, scoring low in agreeableness and high in negative emotionality predicted higher burnout scores. Among Mayo physicians and nurses, reports of emotional distress at work and work-life imbalance were the most consistent predictors of burnout after controlling for personality traits. Among UWEC faculty and instructional staff, fairness at work was the most consistent negative predictor of burnout after controlling for personality.

Introduction

Burnout can be defined as a chronic, negative, work-related psychological state. The characteristics of burnout include three dimensions: emotional exhaustion, depersonalization, and low personal accomplishment. Emotional exhaustion is the most common characteristic presented and it presents itself as a reduction of mental energy. Depersonalization is exhibited as a lack of empathy and a cynical attitude when dealing with clients or patients. The third burnout dimension is when an individual has a negative self-perception, typically with one's own work, known as low personal accomplishment (Lee et al., 2013). Symptoms associated with burnout are physical (e.g., chronic fatigue, headaches), emotional (e.g., feeling cynical and bitter not only about work but life in general, feeling as though you have failed in life), and behavioral (e.g., difficulty concentrating, nightmares) (Rozman et al., 2018).

Although burnout is not a disorder included in the DSM-5, it is a significant concern for employees across professions. Burnout is often high in jobs that are people-oriented, such as human services, educational, and health care occupations. People oriented professions typically require a high level of engagement through personal and emotional contact that can produce

stress (Maslach & Leiter, 2016). Previous research indicates that job stress may diminish an individual's physical health, psychological well-being, and work performance. When individuals are at a critical point where they cannot recover from work demands, stress can cause acute fatigue and increase the risk of burnout.

Burnout is common in many different professions; however, burnout is particularly high in health care, where one in three physicians experience burnout. Burnout is accompanied by job dissatisfaction, increased risk of suicide or drug abuse and dependency, and increased risk of psychopathology. Equally as important is the observation that burnout is linked to lower quality patient care, lower patient satisfaction, decreased levels of patient safety, and greater risk of making medical errors (Brown, Slater, & Lofters, 2019; West, Dyrby, Satele, & Shanafelt, 2012).

The healthcare field is experiencing many changes that may increase employee burnout and jeopardize the prosperity of healthcare organizations (Shanafelt & Noseworthy, 2017). Although organizations are experiencing changes such as fluctuating reimbursement, increased productivity expectation, and large-scale electronic health record adoption, physicians are still required to provide higher quality and more efficient care (Melnick & Powsner, 2016). Organization restructuring can also impact nurses, by increasing their increases in workload, bringing in higher paid nurses who simultaneously have fewer and less-developed skills, and staff changes by replacement of longer tenured nurses with less tenured nurses. A decrease in qualified nurses to care for patients has caused an increase in workload. This can lead to stress due to the increase of patients that need to be cared for at the same number of hours and turnover of patients. Across studies, restructuring and the changes it brings to the organization can lower engagement, reduce morale, increase cynicism and anger (Burke & Greenglass, 2001). Predictably, physicians' and nurses' wellness are critical for empathic, quality care (Melnick & Powsner, 2016).

There is projected shortage of primary care physicians in the near future, which would not only affect fellow physicians but the organization as well. The projected shortage may be due to an increasingly aging U.S. population. Another possible contributor could be an increase in access to medical care with the immediate need for specific specialists while the availability of residency positions decreases. The combination of physician shortage and burnout among physicians could lead to a reduction in clinical hours, low organizational commitment, and intent to leave their medical practice, and the outcome could pose a threat and cost to society (Shanafelt et al., 2016; Maslach & Leiter, 2008). Therefore, it is important to not only address physicians and allied health care workers' burnout but also to recognize it as a system issue.

People fail to realize that anyone can be at risk of developing burnout. The negative effects of burnout can have an impact on many aspects of an individual's life, including their social, home, and work life. Undeniably, most people want to enjoy the work they do and find meaning and purpose in doing it. In the society we live in today, work is essential to survival. Money can't buy happiness, but it can buy security and safety. It not only affects the individual and those around them, but it can also have a negative impact on the organization by decreasing productivity, increasing absenteeism and job abandonment. People who develop burnout are more likely to retire early or abandon their jobs causing the organization to find and train someone new. That is

time, money, and resources the organization has to use, so it is in their interest to prevent burnout among their employees.

Interventions to reduce occupational burnout can increase a person's level of engagement. Engagement is defined as an energetic state of involvement with personally fulfilling activities that enhance one's sense of professional efficacy. Engagement leads researchers to consider the factors in the work environment that enhance individuals' energy, capability, and reliance; promotes absorption with work tasks; and facilitates efficiency and success on the job (Maslach & Leiter, 2008). Therefore, engagement is often viewed as a desirable outcome at work, while burnout is undesirable.

Numerous studies have been conducted to identify potential predictors of burnout, so that corporations can work to mitigate those factors. A particular finding indicates burnout to a certain degree is predicted by individuals' long-standing, maladaptive personality traits that can predispose them to react negatively to work stressors. Specifically, emotional exhaustion is negatively correlated with extroversion and negative emotionality, depersonalization is negatively correlated with disagreeableness and negative emotionality, and personal accomplishments are positively related to extroversion, conscientiousness, and agreeableness, and negatively related to negative emotionality (Ghorpade, Lackritz, & Singh, 2007).

Another line of research has shown the organization and work factors play critical roles in whether employees stay engaged or experience burnout. Specifically, high job demands coupled with low job resources are associated with an increased probability of burnout. Shanafelt and Noseworthy (2016) grouped work factors that are tied to burnout into seven dimensions: workload and job demands, efficiency and resources, flexibility/control over work, work-life integration, organizational values, social support/community at work, and the degree of perceived meaning from work. Although the seven dimensions serve as a good foundation, additional research has documented a host of work factors that also act as drivers of burnout: perceived unfairness and injustice at work, lack of opportunities for growth, lack of challenge, physical demands of the job, the inability to speak up about errors without fearing the consequences, and being given work tasks without enough resources to complete them (Henkens & Leenders, 2010; Carmeli & Gittell, 2008; Maslach & Leiter, 2008). Sincere efforts to prevent burnout must include a personality assessment alongside a comprehensive measure of the many work factors that have been shown to predict burnout.

In the current study, we move beyond what is already known by including both personality and work factors as predictors of burnout; thus, we can measure and hold personality traits constant, before we analyze links between (1) work factors, and (2) burnout and engagement. We predict that personality traits, particularly neuroticism and disagreeableness, will account for a statistically significant proportion of variance in burnout and engagement in both Mayo physicians and nurses as well as our comparison sample of UWEC faculty and instructional staff. Our primary aim, however, is to document in each sample which work factors continue to predict burnout (and engagement) after controlling for employee personality traits.

Method

Participants

Table 1 displays information on the samples. As displayed in Table 1, the majority of both employee samples were between the ages of 30-50, female, and white.

Table 1. *Characteristics of the Samples*

	<i>Mayo Physicians and Allied Health Care Workers</i>	<i>UWEC Faculty and Instructional Staff</i>
Number of Participants	589	140
<i>Age (years)</i>		
Under 30	89 (15.1%)	2 (1.4%)
30-50	356 (60.4%)	77 (55%)
Over 30	140 (23.8%)	60 (42.9%)
<i>Gender</i>		
Women	477 (81%)	83 (59.3%)
Men	103 (17.5%)	52 (37.1%)
Other/Unreported	9 (1.6%)	3 (2.1%)
<i>Race/Ethnicity</i>		
Asian	17 (2.9%)	7 (5%)
Black	3 (.5%)	0
Hispanic/Latino	10 (1.7%)	3 (2.1%)
White	538 (91.3%)	121 (86.4%)
Other/Unreported	19 (3.6%)	9 (6.4%)
<i>Years in Current Position</i>		
0-5	307 (52.1%)	62 (44.3%)
6-10	145 (24.6%)	17 (12.2%)
Over 11	135 (22.9%)	60 (42.9%)
<i>Hours Worked in the Past 7 Days</i>		
Less than 20	30 (5.1%)	4 (2.9%)
49-20	431 (73.2%)	59 (42.1%)
69-50	114 (19.3%)	64 (45.8%)
Over 70	13 (2.2%)	13 (9.3%)
<i>Payment Method</i>		
Salary	242 (41.1%)	136 (97.8%)
Hourly	338 (57.4%)	1 (.7%)
Other/Unreported	9 (1.5%)	2 (2.1%)

Outcome Variables

Burnout. To measure burnout, we used two items from the Maslach Burnout Inventory Manual fourth edition (Maslach, Jackson, & Leiter, 1996-2016) that measure two dimensions of burnout: emotional exhaustion and depersonalization. The items were formulated as statements of work-related feelings (e.g., “I feel burned out from my work,” and “I have become more callous toward people since I took this job”) and were rated on a 7-point scale (ranging from *never* to *every day*). High levels of burnout are established as scores of 4 and above (West et al., 2012).

Engagement. The PERMA-Profilier (Butler & Kern, 2014) includes five pillars of assessment: Positive emotion, Engagement, Relationships, Meaning, and Accomplishment (PERMA). We used three items of the engagement pillar that measure two dimensions of engagement: Absorption and Interest. The items are framed as statements of engagement in the work field (e.g., “At work, how often do you become absorbed in what you are doing?” and “To what extent do you feel excited about and interested in your work?”) on an 11-point scale ranging from never to completely.

Predicted Variables

Personality. The BFI-2 Short (Soto & John, 2017) consists of 30-items that conceptualize personality structure by assessing the Big Five personality factors. Table 2 displays the Big Five statistics for our two samples. The “Big Five” refer to openness (with facets of aesthetic sensitivity, intellectual curiosity, and creative imagination), extraversion (sociability, assertiveness, and energy level), agreeableness (compassion, respectfulness, and trust), conscientiousness (organization, productivity, and responsibility), and negative emotionality (anxiety, depression, and emotional volatility). The items are framed as perceptions of how much one might agree or disagree. Each domain includes positively worded items, for example “Is original; comes up with new ideas”, and negatively worded items of “Has few artistic interests.” The items were rated on a 5-point scale from strongly disagree to strongly agree. The scoring for the negatively worded items were reversed in the data analysis phase.

Table 2. *Descriptive Statistics and Measures of the Big Five Personality Domains.*

Personality Trait	Sample Item	Mayo Clinic		UWEC	
		α	$M(SD)$	α	$M(SD)$
Openness	<i>Is original; comes up with new ideas.</i>	.67	3.61 (0.68)	.74	4.00 (0.68)
Conscientiousness	<i>Is reliable, can always be counted on.</i>	.69	4.25 (0.58)	.76	3.80 (0.74)
Extraversion	<i>Is outgoing, sociable.</i>	.73	3.48 (0.76)	.69	3.20 (0.76)
Agreeableness	<i>Assumes the best about people.</i>	.76	4.23 (0.59)	.74	4.01 (0.65)
Negative Emotionality	<i>Tends to feel depressed, blue.</i>	.77	2.43 (0.76)	.85	2.68 (0.89)

Work Factors

The fourteen work factors included in this study came from a variety of different sources, as shown in Table 3. The fourteen work factors were workload, emotional distress, moral distress, work-life balance, role clarity, role competence, control and autonomy, skill discretion, meaning in work, psychological safety, shared goals and values, positive leader behavior, fairness, and employee-organization values alignment.

Table 3. *Descriptions of Work Factors Measured in the Study.*

Work Factor and Sample Item	Adapted/Derived From	Mayo Clinic		UWEC	
		α	$M(SD)$	α	$M(SD)$
Workload (1-7) <i>How hard did you have to work (mentally and physically) to accomplish your level of performance?</i>	NASA-TLX (Hart, 2006)	.79	5.59 (1.01)	.66	5.45 (0.89)
Emotional Distress (5 items, 1-5) <i>My work puts me in emotionally disturbing situations.</i>	COPSOQ (Pejtersen et al., 2010)	.81	2.86 (0.89)	.78	2.56 (0.80)
Moral Distress (1 item, 0-10) <i>I experience distress at work over having to do things I feel are unethical.</i>	Moral Distress Thermometer (Wocial & Weaver, 2012)	n/a	3.38 (2.95)	n/a	2.66 (2.53)
Work-Life Balance (3 items, 1-5) <i>My current working hours/patterns suit my personal circumstances.</i>	Work-Related Quality of Life scale (Fontinha et al., 2018)	.84	2.97 (1.01)	.82	2.85 (1.02)
Role Clarity (2 items, 0-4) <i>My job responsibilities are clearly defined.</i>	(Royal & Rossi, 1996)	.89	3.44 (0.82)	.84	3.13 (1.05)
Role Competence (3 items, 1-7) <i>I am confident about my ability to do my job.</i>	Psychological Empowerment at Work (Spreitzer, 1996)	.89	6.00 (0.94)	.88	6.03 (0.91)
Control and Autonomy (3 items, 1-7) <i>I have significant autonomy in determining how I do my job.</i>	Psychological Empowerment at Work (Spreitzer, 1996)	.92	4.88 (0.58)	.94	5.63 (1.22)
Skill Discretion (6 items, 1-5) <i>My work provides a variety of challenges.</i>	The Job Content Questionnaire (JCQ) (Karasek et al., 1998)	.73	3.73 (0.58)	.78	3.78 (0.57)
Meaning in Work (3 items, 1-7) <i>The work I do is very important to me.</i>	Psychological Empowerment at Work (Spreitzer, 1996)	.94	5.97 (1.08)	.93	5.86 (1.14)

Psychological Safety (9 items, 1-5) <i>I feel safe being myself at work.</i>	(Edmondson, 1999)	.86	3.66 (0.71)	.90	3.56 (0.86)
Shared Goals and Values (2 items, 1-5) <i>In my work unit, we share a common vision.</i>	(Carmeli & Gittel, 2009)	.91	3.85 (0.80)	.81	3.56 (1.05)
Positive Leader Behavior (9 items, 1-5) <i>My direct supervisor provides helpful feedback and coaching on my performance.</i>	Mayo Clinic Participatory Management Leadership Index (Shanafelt et al., 2020)	.94	3.71 (0.86)	.91	3.65 (1.00)
Fairness (6 items, 1-7) <i>I feel that my job responsibilities are fair.</i>	Distributive Justice (Niehoff & Moorman, 1993)	.87	4.86 (1.30)	.88	4.15 (1.42)
Values Alignment (3 items, 1-5) <i>Our organizational goals and values fit well with my goals and values.</i>	Stanford Values Alignment Scale (Shanafelt et al., 2021)	.87	2.58 (1.06)	.84	2.42 (0.98)

Procedure

We used an anonymous online questionnaire for this study. Data was collected using a secure online survey through Qualtrics. Study researchers sent out an email invitation that included a link to the survey. In the survey, employees answered questions about their 1) level of burnout and engagement at work, 2) perceptions of the imposed demands and available resources of their work, and 3) personality. The survey concluded with a brief demographics section that included questions about participants' current occupation (full-time/part-time, typical hours worked, length of time in current position). This study was approved by the Institutional Review Boards of the participating organizations.

Results

Of the 2,000 physicians and nurses in the Mayo Clinic Northwest Wisconsin region invited to participate, 589 (response rate, 29%) completed the survey. Of the 586 faculty and instructional staff at UWEC and UWEC-Barron County invited to participate, 140 (response rate, 28%) completed the survey. The demographic characteristics of the 589 Mayo Clinic physicians and nurses and 140 UWEC faculty and instructional staff are shown in Table 1. Among the Mayo Sample, 242 (41.1%) are presumed to be physicians indicated by salary pay and 338 (57.4%) are speculated to be nurses given they are hourly pay. The amount of time worked in the last week varied among the two samples, for Mayo Clinic respondents 45% worked less than 40 hours and 55% worked 40 hours or more, whereas 11% of UWEC respondents worked less than 40 hours

and 89% worked 40 hours or more.

Descriptive statistics for burnout are shown in Figure 1. Composite burnout scores (0 to 6) averaged 3.36 (SD= 1.63) for the Mayo Clinic sample (Figure 1A) and 2.97 (SD= 1.63) for the UWEC sample (Figure 1B). Nearly half of Mayo Clinic physicians and nurses, and one third of UWEC respondents, scored high in burnout (scored 4 and above). Responses to individual items for burnout are shown in Figure 2. In both samples (Figure 2A & 2B), responses to the burnout items suggest stronger feelings of burnout than emotional callousness. Descriptive statistics for engagement are shown in Figure 2. Mayo Clinic physicians and nurses reported a moderate level of engagement at work (M= 5.96, SD=1.92). Whereas UWEC faculty and instructional staff experience a moderate to high level of engagement at work (M= 6.77, SD= 1.63).

Figure 1. Descriptive Statistics for Burnout Among Samples.

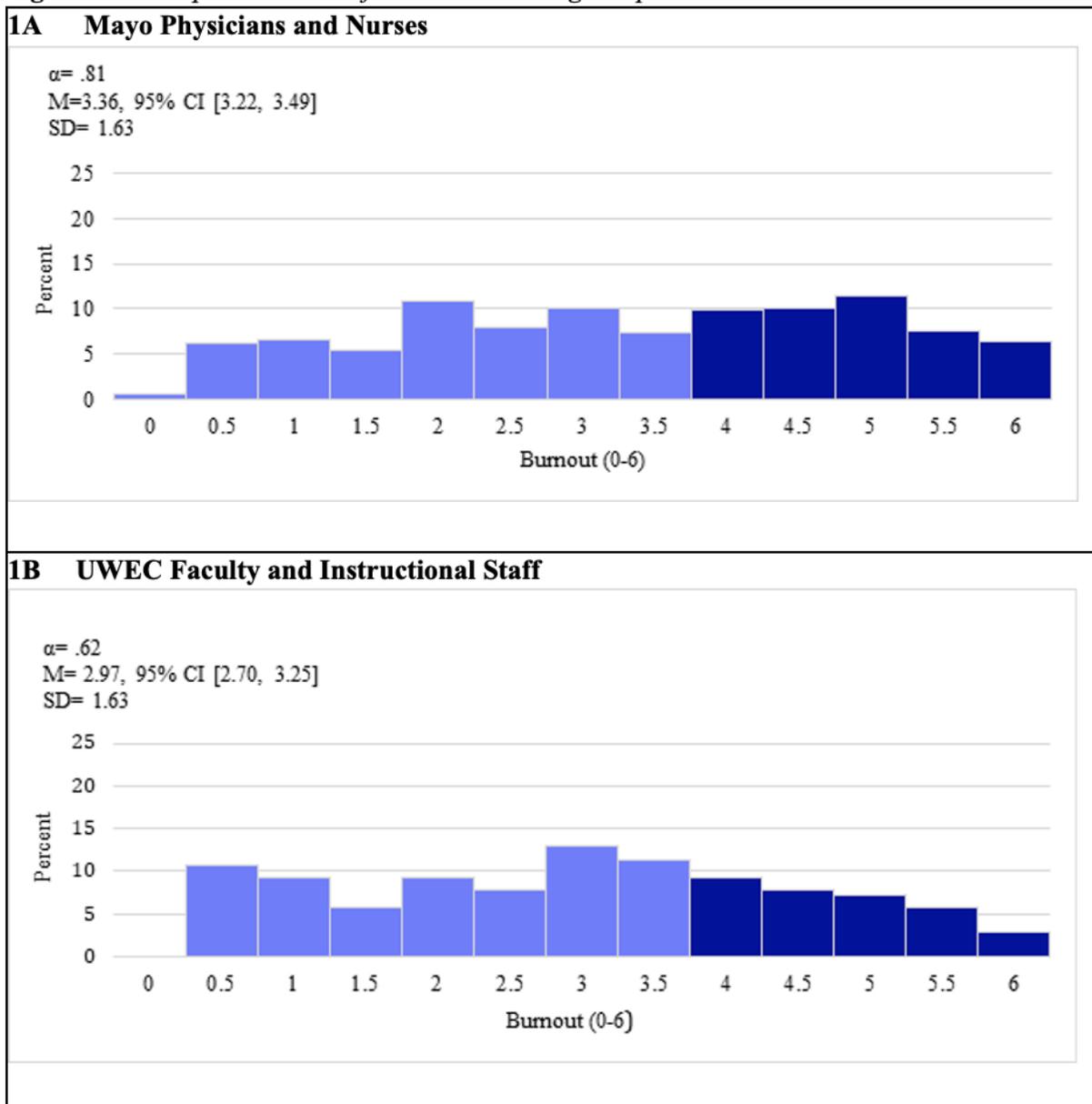


Figure 2. Responses to the Burnout Items.

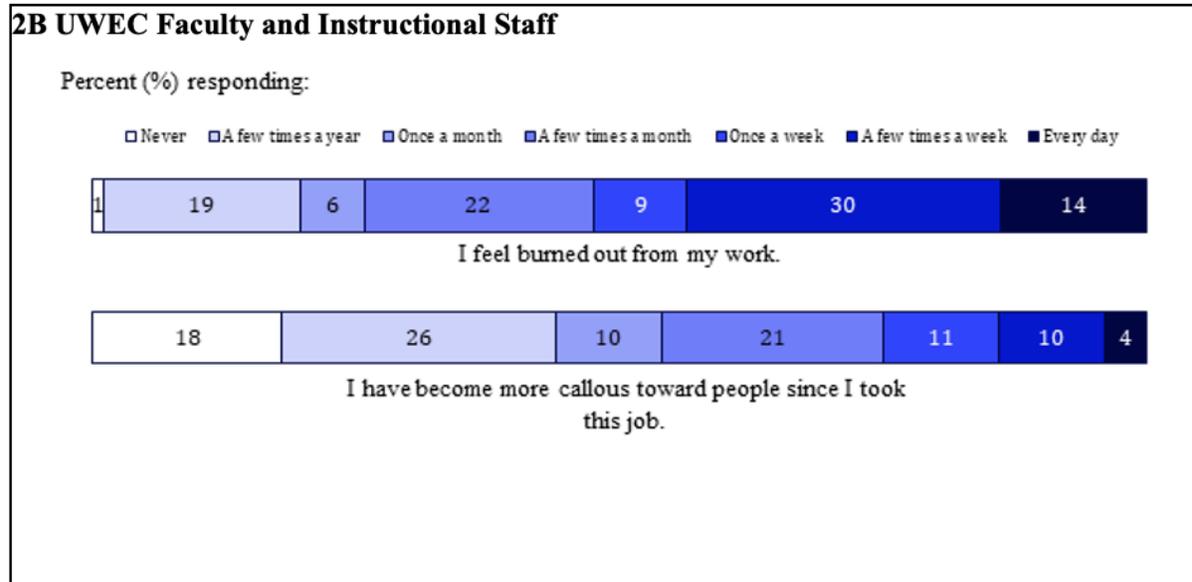
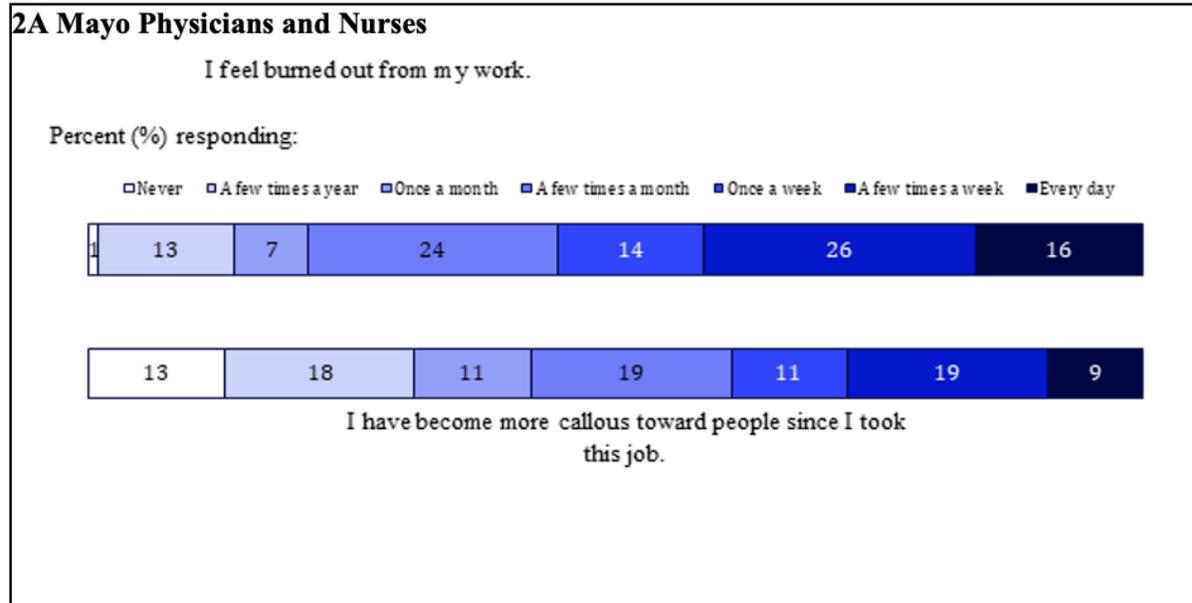
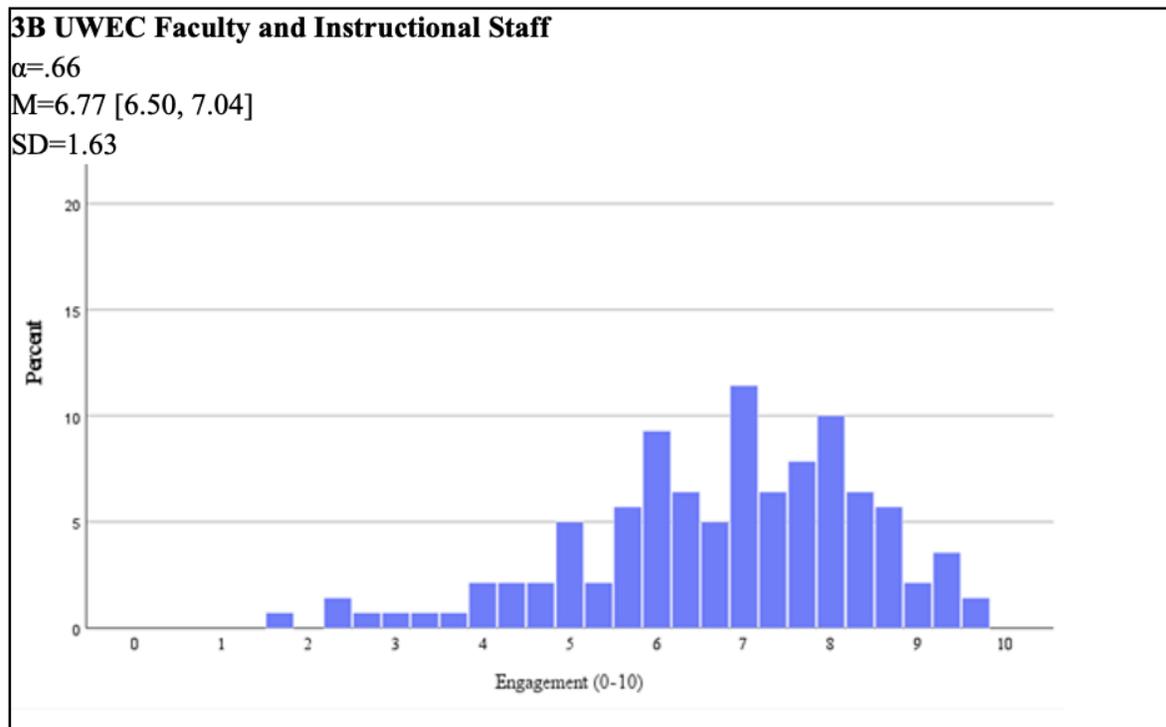
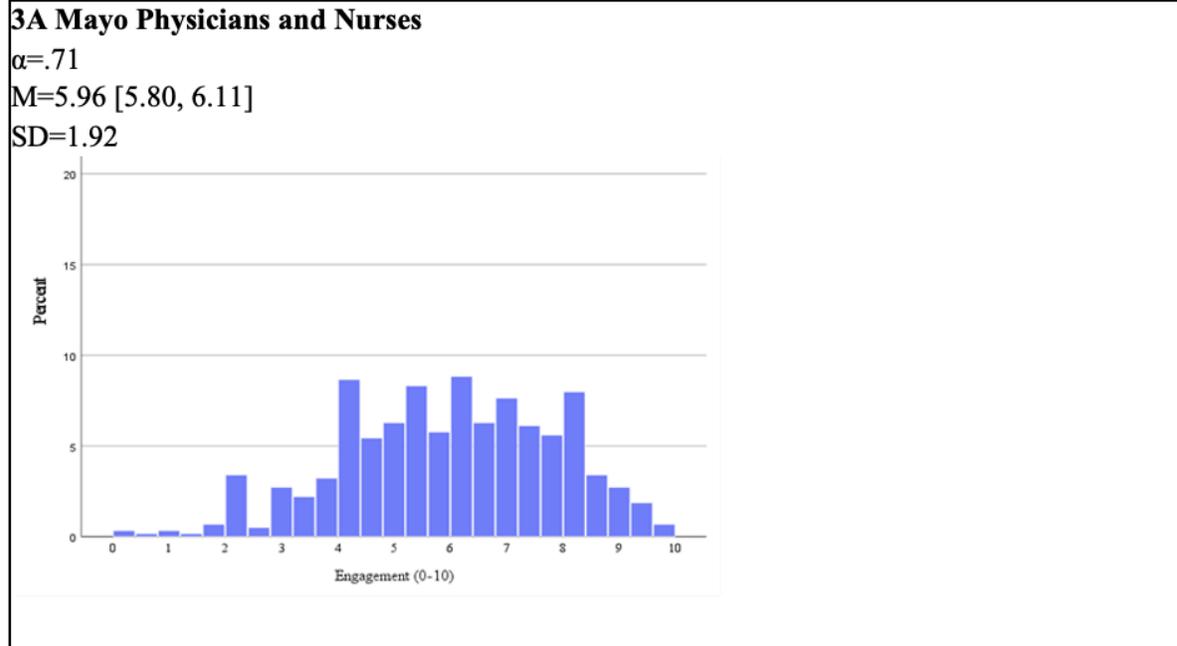


Figure 3. Descriptive Statistics for Engagement Among Samples.



Descriptive statistics for the personality traits measured (1-5 scale) are shown in Table 2. As shown in the table, Mayo Clinic physicians and nurses perceived themselves as quite conscientious and agreeable. UWEC faculty and staff perceived themselves as quite agreeable and open. Both samples scored themselves below the theoretical midpoint for negative emotionality.

Associations between employee personality traits and burnout are shown in Table 4. Employees' personality scores were tied to their level of burnout. In the Mayo sample, all five broad personality factors were tied to burnout. In the UWEC sample, the link between burnout and conscientiousness was similar in magnitude but not statistically; the negative association between burnout and agreeableness and the positive association between burnout and negative emotionality were statistically significant.

Associations between employee personality traits and engagement are shown in Table 5. In the Mayo Clinic sample, all five broad personality factors were tied to engagement. In the UWEC sample, all personality factors except openness were tied to engagement.

Table 4. *Associations Between Employee Personality Traits and Burnout.*

	Mayo (r, 95% CI)	UWEC (r, 95% CI)
Openness	-.10 [-.18, -.02]	.09 [-.06, .26]
Conscientiousness	-.12 [-.20, -.14]	-.15 [-.31, .03]
Extraversion	-.07 [-.15, -.02]	.00 [-.17, .17]
Agreeableness	-.19 [-.27, -.11]	-.30 [-.45, -.12]
Negative Emotionality	.36 [.28, .42]	.41 [.26, .53]

Table 5. *Associations Between Employee Personality Traits and Engagement.*

	Mayo (r, 95% CI)	UWEC
Openness	.15 [.07, .22]	-.03 [-.20, .14]
Conscientiousness	.18 [.10, .26]	.27 [.10, .41]
Extraversion	.18 [.10, .26]	.17 [.01, .33]
Agreeableness	.27 [.19, .34]	.22 [.05, .37]
Negative Emotionality	-.20 [-.28, -.12]	-.25 [-.40, -.09]

The links between work factors and burnout are shown in Table 6 and links between work factors and engagement are shown in Table 7. Employees' ratings of the various work factors were correlated with their levels of burnout and engagement. These are listed in order of magnitude for Mayo Clinic (strongest to weakest regardless of direction).

Table 6. *Links Between Work Factors and Burnout.*

	Correlation with Burnout (<i>r</i>, 95% CI)	
	Mayo Clinic	UWEC
Emotional Distress	.52 [.46, .58]	.49 [.35, .61]
Work-Life Interface	-.50 [-.55, -.43]	-.50 [-.61, -.36]
Fairness	-.47 [-.53, -.40]	-.59 [-.69, -.47]
Values Alignment	-.42 [-.49, -.35]	-.37 [-.50, -.21]
Moral Distress	.37 [.30, .44]	.38 [.23, .51]
Control and Autonomy	-.37 [-.44, -.29]	-.39 [-.53, -.24]
Psychological Safety	-.37 [-.44, -.20]	-.50 [-.62, -.37]
Meaning in Work	-.36 [-.43, -.29]	-.44 [-.56, -.30]
Shared Goals	-.33 [-.40, -.26]	-.53 [-.64, -.40]
Positive Leader Behavior	-.31 [-.38, -.23]	-.40 [-.53, -.26]
Role Clarity	-.28 [-.35, -.20]	-.44 [-.56, -.29]
Workload	.26 [.18, .33]	.24 [.07, .39]
Skill Discretion	-.24 [-.32, -.16]	-.37 [-.50, -.22]
Role Competence	-.22 [-.29, -.14]	-.17 [-.33, .00]

Table 7. *Links Between Work Factors and Engagement.*

	Correlation with Engagement (<i>r</i>, 95% CI)	
	Mayo Clinic	UWEC
Meaning in Work	.56 [.50, .61]	.51 [.38, .63]
Skill Discretion	.40 [.33, .47]	.31 [.15, .45]
Values Alignment	.38 [.31, .45]	.22 [.06, .37]
Fairness	.36 [.29, .43]	.21 [.05, .37]
Shared Goals	.35 [.28, .42]	.30 [.14, .45]
Psychological Safety	.33 [.26, .40]	.25 [.08, .40]
Work-Life Interface	.28 [.20, .35]	.23 [.06, .38]
Role Competence	.27 [.19, .34]	.18 [.01, .33]
Positive Leader Behavior	.27 [.19, .34]	.22 [.06, .37]
Emotional Distress	-.26 [-.34, -.19]	-.09 [-.25, .08]
Role Clarity	.25 [.17, .32]	.21 [.05, .37]
Control and Autonomy	.24 [.17, .32]	.13 [-.04, .29]
Moral Distress	-.23 [-.30, -.15]	-.12 [-.28, .05]
Workload	.06 [-.02, .14]	.11 [-.06, .27]

Regression models predicting burnout among Mayo Clinic physicians and nurses is shown in Table 8 and regression models predicting burnout among UWEC faculty and staff can be found in Table 9. We regressed burnout on the five personality dimensions and fourteen work factors. In subsequent models, we added work factors one by one (model by model), in order of the magnitude each work factor correlated with burnout. We continued this process until the addition of any given work factor no longer accounted for additional variance in the prediction. The included tables capture the results of these analyses. The final model for the Mayo Clinic sample accounted for 49% of the variance in burnout ($R^2 = .485$). Of the five personality traits in the Mayo sample, two significantly accounted for 14% of the variance in levels of burnout ($R^2 = .14$): Agreeableness and Negative Emotionality. After controlling for personality reports of emotional distress at work and work-life imbalance were the most consistent predictors of burnout among the Mayo clinic sample ($p < .001$). Other work factors that contributed, but less consistently, were perceptions of Justice/Fairness, Values Alignment, Meaning in Work, and Workload. In the final model for the UWEC sample, 49% of the variance accounted for burnout. The five personality traits accounted for 21% of the variance in levels of burnout ($R^2 = .21$). Agreeableness was negatively correlated, and Negative Emotionality was positively correlated and were statistically significant independent predictors. After controlling for personality, perceived fairness at work was a consistent negative predictor of burnout in the UWEC sample ($p < .001$). Other work factors that contributed, but less consistently, were perceptions of Shared Goals and Meaning in Work.

Table 8. Regression Results: Predicting Burnout among Mayo Clinic Physicians and Nurses.

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	β	p												
Openness	-.03	.407	-.098	.005	-.086	.010	-.078	.020	-.076	.022	-.060	.065	-.058	.073
Conscientiousness	-.01	.847	.021	.564	.050	.156	.042	.223	.043	.217	.051	.132	.038	.262
Extraversion	.07	.080	.054	.138	.038	.274	.031	.373	.035	.305	.069	.046	.064	.062
Agreeableness	-.10	.019	-.071	.049	-.081	.018	-.075	.028	-.063	.067	-.030	.373	-.037	.273
Negative Emotionality	.35	<.001	.248	<.001	.210	<.001	.207	<.001	.208	<.001	.209	<.001	.203	<.001
Emotional Distress			.484	<.001	.364	<.001	.332	<.001	.320	<.001	.315	<.001	.282	<.001
Work-Life Interface					-.295	<.001	-.236	<.001	-.227	<.001	-.223	<.001	-.205	<.001
Fairness							-.124	.003	-.068	.139	-.045	.324	-.031	.486
Values Alignment									-.109	.008	-.076	.062	-.087	.033
Meaning in Work											-.176	<.001	-.198	<.001
Workload													.099	.005
ΔR^2	.144		.226		.071		.008		.007		.023		.006	
Total R^2	.144		.370		.441		.449		.456		.479		.485	

Table 9. Regression Results: Predicting Burnout among UWEC Faculty and Instructional Staff.

	Model 1		Model 2		Model 3		Model 4	
	β	<i>p</i>	β	<i>p</i>	β	<i>p</i>	β	<i>P</i>
Openness	.095	.231	.062	.354	.047	.471	.034	.597
Conscientiousness	.046	.605	.068	.368	.053	.475	.055	.454
Extraversion	.064	.428	.015	.823	.024	.725	.042	.534
Agreeableness	-.193	.022	-.126	.077	-.097	.171	-.066	.348
Negative Emotionality	.373	<.001	.285	<.001	.258	.001	.264	<.001
Fairness			-.502	<.001	-.391	<.001	.363	<.001
Shared Goals					-.211	.009	-.157	.058
Meaning in Work							-.168	.024
ΔR^2	.212		.232		.028		.020	
Total R^2	.212		.444		.472		.492	

Regression models predicting engagement among Mayo Clinic physicians and nurses are shown in Table 10 and regression models predicting engagement for UWEC faculty and instructional staff are shown in Table 11. Burnout regression steps were replicated for the regression analysis of engagement in both samples. The final model for the Mayo Clinic sample accounted for 39% of the variance in engagement scores ($R^2 = .393$). Of the five personality traits, two significantly accounted for the variance in levels of engagement ($R^2 = .11$): Extraversion was positively correlated, and Agreeableness was negatively correlated. After accounting for personality traits, the most consistent predictors being Meaning in Work ($p < .001$), Skill Discretion ($p = .008$), and Fairness ($p = .016$). Other work factors that contributed, but less consistently, were perceptions of Values and Alignment, and Shared Goals. The final model for the UWEC sample accounted for 32% of the variance in engagement scores ($R^2 = .318$), with the only independent predictors being Conscientiousness ($p = .048$) and Meaning in Work ($p < .001$).

Table 10. Regression Model: Predicting Engagement among Mayo Nurses and Physicians.

	Model 1		Model 2		Model 3		Model 4		Model 5		Model 6		Model 7	
	β	<i>p</i>												
Openness	.073	.073	.025	.477	.026	.467	.027	.437	.025	.457	.023	.494	.035	.312
Conscientiousness	.069	.109	.037	.327	.024	.517	.027	.460	.027	.448	.026	.464	.021	.550
Extraversion	.088	.042	.003	.943	.002	.962	.015	.689	.032	.381	.036	.330	.035	.334
Agreeableness	.200	<.001	.086	.023	.082	.027	.071	.054	.079	.030	.079	.030	.079	.030
Negative Emotionality	-.085	.052	-.060	.114	-.055	.144	-.039	.294	-.024	.520	-.016	.665	-.009	.815
Meaning in Work			.507	<.001	.434	<.001	.410	<.001	.391	<.001	.382	<.001	.377	<.001
Skill Discretion					.167	<.001	.103	.011	.105	.009	.096	.017	.108	.008
Values Alignment							.167	<.001	.078	.086	.062	.175	.049	.288
Fairness									.152	<.001	.131	.003	.109	.016
Shared Goals											.080	.044	.066	.101
Emotional Distress													-.077	.048
ΔR^2	.109		.219		.021		.021		.014		.004		.004	
Total R^2	.109		.329		.350		.371		.385		.389		.393	

Table 11. Regression Model: Predicting Engagement among UWEC Faculty and Instructional Staff.

	Model 1		Model 2	
	β	p	β	p
Openness	-.071	.396	-.014	.855
Conscientiousness	.167	.079	.166	.048
Extraversion	.105	.224	.064	.401
Agreeableness	.151	.088	.024	.768
Neuroticism	-.098	.321	-.066	.451
Meaning in Work			.469	<.001
ΔR^2	.122		.196	
Total R^2	.122		.318	

Discussion

Personality alone accounted for roughly 14% of the variance in burnout among Mayo Clinic employees and roughly 21% of the variance in burnout among UWEC faculty and instructional staff. This pattern is also exhibited for engagement, where personality alone accounted around 11% in the Mayo Clinic sample and 12% in the UWEC sample. These findings demonstrate the importance of accounting for personality when measuring levels of burnout and engagement. In both samples, individuals who are lower in agreeableness and lower in negative emotionality are more likely to express high levels of burnout. For engagement, only extraversion and agreeableness contributed to the variance in the Mayo Clinic sample.

An additional 35% of the variance in burnout in the Mayo Clinic sample and an additional 28% of the variance in burnout in the UWEC sample was explained by contributing work factors. In the Mayo Clinic sample, after controlling for personality, reports of emotional distress at work and work-life imbalance were the most consistent predictors of burnout. For the UWEC sample, after controlling for personality, perceived fairness at work was a consistent negative predictor of burnout. An additional 28% of the variance in engagement in the Mayo Clinic sample and an additional 20% of the variance in engagement in the UWEC sample was explained by work factors. In the Mayo Clinic sample, the most consistent predictors of engagement are Agreeableness, Meaning in Work, Skill Discretion, and Fairness. In the UWEC sample, the only independent predictors were conscientiousness and meaning in work.

Mayo Clinic has a strong history of organizational efforts to combat burnout and enhance engagement (Shanafelt & Noseworthy, 2017; DeChant et al., 2019; Dieser, Edginton, & Ziemer, 2017). We know of no parallel efforts out of UWEC for their faculty and instructional staff. Perhaps the university prioritizes students' well-being over that of faculty and instructional staff. However, students' and patients' quality of care and learning might actually have strong parallels. If faculty and instructional staff are more engaged and less burned out, they might display higher quality teaching as well as compassion toward their students, both of which would likely foster better learning. Further investigation is needed to get a better understanding of the outcome associated with faculty and instructional staff burnout throughout the educational system.

Our results suggest a need for strategies to mitigate burnout in health care as well as in higher education, and they also direct our attention to strategies that may be most effective. While we do not discourage individuals from personally working to strengthen specific work resources highlighted by these analyses, we do think that the nature of the work factors shown by our analyses to predict burnout implies the need for institution-level solutions.

For example, focus groups could be used to identify the specific areas from which negative perceptions of workplace conditions are originating. At Mayo, these focus groups would likely want to attend to the specific situations in which physicians and nurses feel that they are being placed under levels of emotional distress that fall outside of their general job description. Mayo could then hold sessions that could teach employees about ways in which they can better manage and cope with their emotional distress as it was reported in the previously held focus groups. Focus groups at UWEC could attend to the ways in which faculty and instructional staff feel that what is being asked of them is unfair. UWEC could then implement a way for faculty to semi-anonymously report instances in which they believe that they were treated unfairly.

Previous research lends support to the idea that the issues affecting work-life balance likely stem from the fact that skilled nurses and physicians have recently been in short supply despite increasing demands for quality healthcare amidst the COVID-19 pandemic. Additionally, the limited number of professionals who *are* available to give treatment increasingly lack the skills necessary to deliver effective care. Tentatively, a solution for this issue could be waitlisting patients based on the urgency of their presenting issues. This would mean that potentially fewer patients would receive care, but it might decrease the workload placed upon any one physician or nurse, which could reduce burnout and promote a higher quality of care.

Our data were collected at an unusual time in global history. It is possible that levels of burnout were higher than they would have been prior to the COVID-19 pandemic, especially in the case of Mayo physicians and nurses on the front lines of patient care. A possible future direction would include the associated differences between physicians and nurses using the same study design used in this study. Additionally, this data might look very different a few years down the line as the United States continues to move past the COVID pandemic. Additionally, the work factors that are less predictive of burnout today may later rise to the top amidst evolving economic and occupational conditions.

In conclusion, we have shown that employees who are relatively high in disagreeableness and negative emotionality are at increased risk of burnout. Yet, even after accounting for those individual risk factors, specific aspects of the work environment, notably *fairness* and *work-family balance*, contribute importantly to the prediction of burnout, and *meaning in work* contributes importantly to the prediction of engagement. Mayo Clinic and UWEC should put concerted effort toward enhancing these work factors for their employees, thereby reducing burnout, and enhancing engagement, and thereby potentially enhancing patient care and student learning.

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Ciliary protein localization in *Caenorhabditis elegans*: a review exploring *gar-3* as an additional localization factor

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Abstract

gar-3, a gene that encodes a G protein-coupled acetylcholine receptor, plays a role in both neurotransmission and protein localization to synapses. We have begun to ask whether *gar-3* affects the localization of PKD-2 protein to primary cilia in *C. elegans*, a free-living nematode. Dysregulated PKD-2 localization has been linked to polycystic kidney disease in humans. Thus, understanding the factors that mediate PKD-2 protein localization to cilia might help us better understand the molecular basis of polycystic kidney disease. We chose to utilize *C. elegans* as our model organism as it is transparent and has cilia structure similar to that of mammalian kidney cells. In order to address our question, we use both genetic and reverse genetic techniques to reduce *gar-3* gene expression in *C. elegans* transgenic for PKD-2::GFP. These techniques allowed us to utilize fluorescent microscopy to assess PKD-2 localization to primary cilia in a living animal. In this paper, we describe our research in the context of cilia biology, polycystic kidney disease and what is known about *gar-3*.

Understanding ADPKD by Studying Model Organisms

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by fluid filled sacks (cysts) within the kidneys. These cysts crowd out the healthy tissue and ultimately contribute to kidney failure. Mutations in the genes, PC1/PKD-1 and PC2/PKD-2, which encode polycystin-1 and polycystin-2 respectively, cause most cases of ADPKD. The polycystins act as co-receptors and localize to primary cilia of human kidney epithelial cells where they are thought to sense fluid flow. In addition to the changes in polycystin localization and expression, ADPKD involves changes in gene expression of many other genes. Identification of these changes in gene expression may lead to a better understanding of the molecular pathways underlying ADPKD.

ADPKD belongs to a category of genetic diseases classified as ciliopathies; the underlying cellular defect in these diseases is dysfunctional, malformed, or absent cilia. Cilia are projections from the cell membrane and aid in both movement of the cell or fluids and transmission of signals to the cell. Motile cilia are able to move on their own while primary cilia lack the mechanism for intrinsic movement. Cilia are found on nearly all cells in the human body and play different roles depending on their location. For example, cilia lining the renal epithelia in the kidneys may act as flow sensors for urine while cilia in the developing heart play a vital role in the establishment of left-right asymmetry (Dasgupta and Amack 2016). In the Lyman Gingerich lab, we study the relationship between polycystin-2 localization, gene expression, cilia

function, and PKD using 2 model organisms: *C. elegans* and zebrafish.

Although the excretory system of *C. elegans* is not comparable to that of humans, aspects of ADPKD can be studied using *C. elegans* as a model. Primary cilia are found on cell surfaces of many different eukaryotic cells, including human kidney epithelial cells. By contrast, the only ciliated cells in *C. elegans* are a subset of neurons; thus, *C. elegans* can survive without functional cilia while vertebrates cannot. *C. elegans* are used as a model organism for studying ADPKD because the PKD-2 protein localizes to the primary cilia of some of their sensory neurons; this is similar to PKD-2 protein localization to cilia of human kidney epithelial cells. Thus, ciliary protein localization can be easily assessed in *C. elegans*. By tagging PKD-2 with the green fluorescent protein, GFP, the localization of PKD-2 to specific cilia in *C. elegans* can be easily assessed using a fluorescent microscope (Scholey et al. 2004 and Bae et al. 2008).

In order to explore cystic kidney disease in an organism with a kidney more similar to that of humans, we look to the zebrafish. Larval zebrafish have a pronephros (developing kidney) that is similar in structure and patterns of gene expression to the nephron of the adult human kidney. In addition, the pronephros is functional as it develops, and a number of cystic kidney mutants have been identified (Poureetezadi and Wingert 2016). This means that the zebrafish is a viable organism to study polycystic kidney disease and changes in gene expression as cyst development progresses. Our lab has found a number of genes with altered expression in a zebrafish model of cystic kidney disease (our own unpublished data). Because *C. elegans* provide a simpler system for assessing some aspects of cilia form and function, we turn to *C. elegans* for analysis of individual genes. This review will focus on methods of altering gene expression and analyzing PKD-2::GFP localization in *C. elegans* to provide background for analysis of *chr5a/gar-3*.

chr5a gene expression has been observed in fish at synaptic junctions and in the retinal pigmented epithelium, a ciliated layer of cells in the eyes. *chr5a* encodes a muscarinic receptor that has G-protein-coupled acetylcholine receptor activity. In zebrafish larva with cystic kidneys, we observed a decrease in *chr5a* expression, compared to wild-type larva (our own unpublished data). There could possibly be a link between the decreased expression of the *chr5a* gene in mutant zebrafish and cystic kidneys. Our lab is currently investigating the role of *gar-3*, the *C. elegans* ortholog of *chr5a*, in PKD-2 ciliary localization using genetic and reverse genetic approaches. We are using PKD-2::GFP localization as an indicator for PKD-related cilia defects.

The pattern of PKD-2::GFP mislocalization can indicate different types of abnormalities of the cilia. For example, the *cil* genes in *C. elegans* were identified and categorized based on their effects on PKD-2::GFP localization (Bae et al., 2008). Six mutants were placed in together because they had abnormal distribution of PKD-2 in the dendrites; these genes influence how PKD-2::GFP is targeted to specific subcellular locations. Additionally, these mutants could possibly affect vesicular transport, PKD-2 localization in the endoplasmic reticulum, and PKD-2::GFP in the cilium (Bae et al., 2008). Five additional mutants were placed in a separate group, because the PKD-2::GFP localization within the cilium proper was abnormal compared to wild type. Thus, genes in this second group may specifically regulate PKD-2 localization and maintenance within the cilium itself (Bae et al., 2008).

These mislocalization patterns in both groups were concurrent with dysfunctional cilia as evidenced by male mating behavior and olfaction assays. Thus, not only the localization of PKD-2 to the cilia but also the subciliary localization and amount of protein localized each has an important effect on cilia form and function. In addition, PKD-2::GFP mislocalization may be indicative of 1) specific defects in the trafficking of the protein, 2) defects in the structure of specific primary cilia, or 3) a more generalized defect in all primary cilia. To determine which is occurring, male mating behavior and olfaction assays are frequently combined with assessments of cilia structure to broaden the scope of cilia assessed.

Male mating behavior in *C. elegans*, is regulated by the male-specific ciliated neurons to which PKD-2::GFP localizes. The mating analysis techniques used in Bae et. al, 2008 assessed response behavior efficiency and mating efficiency because PKD-2 affects the sexual attraction to females, the ability to locate the vulva, and the active response to a potential mate. The results of these analyses demonstrated that mating behavior may not be solely dependent on PKD-2::GFP localization patterns as four mutants, *cil-2(my2)*, *my9*, *cil-3(my11)*, and *my14*, had normal male mating behavior. Additionally, two mutants, *cil-1(my15)* and *cil-5(my13)*, have normal male mating behavior but cannot reproduce successfully. Therefore, a mutation in one of the *cil* genes can result in defects in fertility, copulation, and/or ciliogenesis in addition to PKD-2::GFP mislocalization.

PKD-2::GFP mislocalization can be due to 1) specific defects in the trafficking of the protein, or 2) the structure of specific primary cilia, or 3) a more generalized defect in all primary cilia. To assess whether other primary cilia are also affected, behaviors dependent on other ciliated neurons can be analyzed. For example, *bbs-7(my13)* mutants have both abnormal mating behavior responses and abnormal chemotaxis to isoamyl alcohol but not diacetyl or benzaldehyde (Braunreiter et al. 2014). Perception of each of these chemicals involves a different set of ciliated neurons.

Dye-filling assays can be used to assess the structural integrity of neurons exposed to the external environment through ciliated endings. For example, in *cil-5(my13)* animals, amphid neurons fill weakly while phasmids are never fill with DiI. On the other hand, *xbx-1(my17)* animals are completely dye-filling defective, indicative of more global cilia defects (Bae et al., 2008).

When working in vertebrate kidney cells, a number of additional techniques can be employed. For example, immunolocalization of PC1 and PC2, Ca²⁺ microfluorimetry and generation of affinity-purified antibodies were common methods used to analyze flow-induced Ca²⁺ signaling in embryonic kidney cell cultures (Nauli et. al., 2003). Primary cilium are needed to detect fluid flow in cells, which is required for flow-induced Ca²⁺ signaling. Using mutated embryonic kidney cell cultures, short or no cilia formed with decreased expression of PC1 after spending one day in a medium that promoted cell division. Therefore, a mutation in PC1 affects production of cilia and is required for flow-induced Ca²⁺ signaling. However, Nauli and colleagues also found that using thrombin could increase cytosolic calcium in both mutant and wild-type cells indicating that the defects in the mutant cells were not due to an inability to move Ca²⁺, but a specific defect in sensing fluid flow (2003).

***chr5a/gar-3* function**

chr5a/gar-3 encodes a muscarinic receptor in zebrafish and *C. elegans*, respectively. Initial characterization of this family of receptors in zebrafish involved cloning, phylogenetic analysis, and immunohistochemistry methods to analyze protein localization. The six genes identified were *chr1a*, *chr1b*, *chr3a*, *chr5a*, and *chr5b*, also known as the *chr*-odd genes. Using RT-PCR, total RNA count was quantified for four different developmental stages revealing that all genes were expressed at the metamorphic and adult stages, whereas only *chr1a*, *chr3b*, and *chr5b* were expressed during the larval stage. These differences in timing of expression may reveal that the different genes have acquired different roles in development (Nuckels et. al., 2011).

Although we are uncertain how loss of *chr5a/gar-3* expression might result in cystic kidneys and whether the link might involve changes in cilia receptor localization or cilia structure, we are curious whether this gene might be related to cystic kidney disease. Our approach mirrors that taken with *bbs-7(my13)* and *xbx-1(my17)*: to downregulate *gar-3* gene expression and assess both PKD-2::GFP localization specifically and cilia structure and function more globally in *C. elegans*.

Methods and Primary Results

Unlike the research involving *my13* and *my17*, we plan to use both forward and reverse genetic techniques. To produce *C. elegans* which are both homozygous for a mutation in the *gar-3* gene and express PKD-2::GFP, we will cross strain VC657 with PT443. This will also enable us to examine males homozygous for the mutation in *gar-3*. Our second approach involves reducing *gar-3* mRNA using RNA interference.

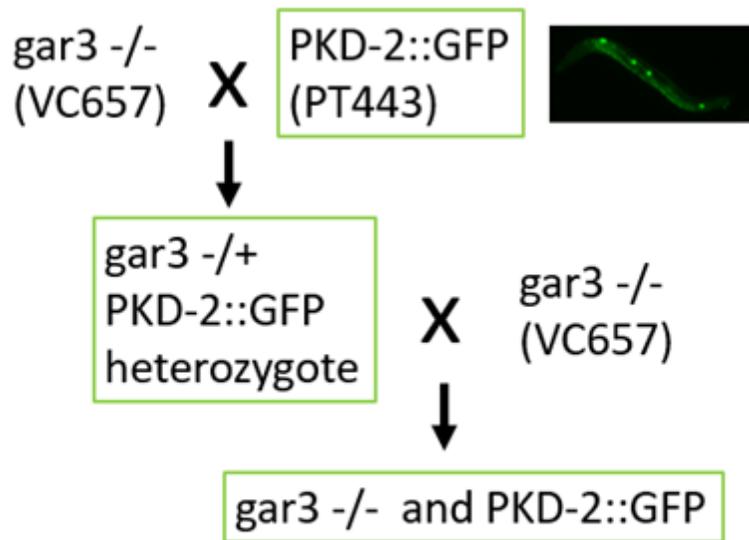


Figure 1. Genetic crossing scheme to analyze PKD-2::GFP localization in *gar3* mutant animals.

With both approaches, to analyze PKD-2::GFP localization to ciliated neurons, male progeny are mounted onto agar pads atop microscope slides with levamisole to prevent movement. *C. elegans* males are analyzed under the 100x oil objective with a GFP filter and UV light. Male-specific neurons in the head and tail normally contain PKD-2::GFP in the cell bodies and the cilia but not in the dendrites connecting the cell bodies to the cilia. In our initial analysis, five of six males homozygous for the mutation in *gar-3* lacked PKD-2::GFP expression in the male-specific tail neurons and exhibited exaggerated head movements. We have not yet done further analysis of the tail neurons to see if the mutation causes defects in the neurons themselves.

Conclusion

The zebrafish and *C. elegans* systems provide complementary advantages to efforts to understand the biology of cystic kidney diseases. Although the zebrafish pronephros is more similar to the human kidney, the optical transparency and accessibility of *C. elegans* cilia, combined with similarities in cilia structure and function, make both effective tools for understanding cilia and, potentially, the role of *gar-3*. Since our study was limited by our small sample size, we cannot draw conclusions about the effects decreased expression of the *gar-3* gene has on protein localization. Moving forward, experiments involving *gar-3* genetic variants including as strains VC670 and JD217 will be explored in addition to the VC657 variant.

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Mental Health Clinicians' Experiences in Transitioning to Telehealth Service Delivery During the COVID-19 Pandemic

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Abstract

The COVID-19 pandemic necessitated an abrupt, unexpected shift of outpatient mental health services to Internet-based telehealth platforms. This study sought to explore how mental health clinicians experienced this dramatic change in their mode of service delivery. The study addressed the following research questions: (1) Were there significant changes in mental health clinicians' compassion satisfaction and symptoms of burnout between the outset of the COVID-19 pandemic and one year later? (2) Was there a significant shift in clinicians' comfort/discomfort with telehealth technology between the outset of the pandemic and one year later? (3) How do clinicians perceive the positive and negative aspects of online service-delivery? (4) To what degree do clinicians experience challenges related to establishing eye contact and detecting subtle nonverbal cues during telehealth sessions? An electronic survey was administered to a convenience sample of clinicians from four regional mental health clinics in northwestern Wisconsin. The Professional Quality of Life Scale (ProQOL) (Stamm, 2010) was used to measure clinician compassion satisfaction and symptoms of burnout. In addition, the researchers developed qualitative survey items to more fully explore clinicians' experiences during the transition to telehealth due to the pandemic. Paired samples t-tests were used to evaluate changes in compassion satisfaction and burnout before and one year after the transition to online service delivery. Qualitative methods were used to analyze responses to the open-ended survey questions. It was hypothesized that the abrupt transition to telehealth service delivery would be associated with decreased compassion satisfaction and increased risk of burnout. The results of paired samples t-tests suggested support for both hypotheses. Specifically, clinicians reported that their compassion satisfaction at one year into the pandemic was lower than immediately before the onset of the pandemic, and they reported more symptoms of burnout one year into the pandemic than before the pandemic began. Findings regarding clinicians' comfort with telehealth, positive and negative perceptions of online service delivery, and challenges with eye contact and detection of subtle nonverbal cues are also reported.

Review of Literature

General Introduction

The COVID-19 pandemic has necessitated sudden and sweeping changes to the delivery of outpatient mental health treatment. Because the pandemic has been unprecedented in this regard, no corpus of literature from prior research on similar situations was available at the time this study was initiated. The available literature regarding the key variables of interest and

the definition, history, and positive and negative attributes of Internet-based health services in general, serves as the foundation for this study.

Online mental health services offer clients an additional avenue through which to access treatment. Internet-based mental health services allow clients to speak with their mental health professional from the convenience and comfort of their own home (or other private location) through the use of a computer, tablet, or smartphone. The delivery of mental health services via technology is referred to in the literature by numerous names, including “online therapy,” “e-therapy,” “telemental health,” “telehealth,” and “Internet-based therapy.” Internet-based mental health treatments have been found to be effective in the treatment of numerous mental health conditions including obsessive-compulsive disorder (Brand & McKay, 2012), anxiety disorders, bipolar disorders (DelliFrane & Dansky, 2008), substance abuse (Bensink et al., 2006), depression and schizophrenia (Bensink et al., 2006; DelliFrane & Dansky, 2008).

Mental health providers are primarily concentrated in larger urban areas across America; less than 7% of all psychiatrists live in rural areas (Hartley et al., 2002). In areas with the lowest numbers of mental health providers, only 41% of Americans with a diagnosed mental illness reported receiving any treatment in 2014 (Substance Abuse and Mental Health Services Administration [SAMHSA], 2015). With this scarcity of mental health providers in many outlying areas, Internet-based mental health services are invaluable to clients who might not otherwise have feasible access to a provider.

An Abbreviated History of the Use of Technology for Mental Health Service Delivery

The use of technology to deliver mental health services began in 1955 at the Nebraska Psychiatric Institute when Van Lear Johnson began providing group psychotherapy and psychiatric consultation via closed-circuit television (Institute of Medicine, 1996). The use of early forms of telehealth continued through the 1960s, but in those early stages, the hardware was expensive and difficult to use, and services remained very limited geographically because the technology could only reach distances up to 20 miles (Dwyer, 1973). In 1968, Veterans Administration (VA) hospitals throughout Nebraska began providing the first interhospital online therapy; other VA hospitals soon developed the capacity to connect online with the central resources available at Massachusetts General Hospital (Godleski et al., 2008).

By the 1990s, technology-based mental health service delivery had become much more efficient and advanced, enabling mental health professionals to provide virtual medication management services and conduct virtual patient evaluations, treatment planning, and both individual and group psychotherapy (Smith & Allis, 1998). As computers and Internet service became available to the public, the delivery of mental health services via technology became increasingly prevalent, though it was still available only to a small subset of affluent Americans. The Telecommunications Act of 1996 mandated that high-speed Internet service be made available to citizens in rural areas at costs comparable to those in urban areas (Adams et al., 2018). Although this legislation was an important step forward, it remains the case – even today – that many Americans cannot access online mental health services due to their inability to afford Internet service and/or electronic devices such as computers, tablets, and cell phones.

Benefits and Limitations of Online Therapy

Prior research has identified both benefits and concerns regarding the delivery of mental health services via the Internet. In terms of benefits, telehealth technology provides access to mental health treatment for many clients who may feel uncomfortable with in-person sessions (Simpson et al., 2005), or who live in geographic areas that lack any mental health service providers (Hilty et al., 2007). Online service provision has the potential to improve client attendance and satisfaction by reducing or eliminating travel (Simpson et al., 2005). The telehealth option can provide needed treatment for clients who may be unable to attend in-person sessions due to physical or psychiatric disability (Institute of Medicine, 1996). The ability to provide services online also benefits clinicians, by reducing or eliminating travel-time, and by making it feasible for them to work from home if they have childcare or eldercare responsibilities.

Research has yielded evidence that telehealth services can help to prevent impatient rehospitalization and can improve compliance and overall satisfaction with treatment (Godleski et al., 2012). Simpson and Reid (2014) reviewed 23 studies which found that the quality of therapeutic alliance between the client and counselor in telehealth is comparable to in-person psychotherapy. Systematic reviews of service delivery via telehealth have shown that its efficacy when used for assessment, diagnosis and treatment is comparable to in-person service delivery. Some studies have suggested telehealth may even be more effective for treating older children, adolescents, adults, and clients with differing cultural backgrounds (Hilty et al., 2013; Molfenter et al., 2015).

Conversely, there are also several concerns regarding Internet-based psychotherapy. One such concern is related to protecting the security of sensitive client mental health information. Practitioners may experience discomfort about offering online therapy (as compared to in-person therapy) due to concerns about client privacy and confidentiality, security of telehealth sessions, and potential professional liability in the event of a data breach in which protected health information is inadvertently disclosed to unauthorized entities.

Health Insurance Portability and Accountability Act (HIPAA) Rules for Telehealth

The privacy of patient health records in general was first formally protected by the Health Insurance Portability and Accountability Act (HIPAA) of 1996. In 2003, HIPAA rules were expanded to address the protection of *electronic* health information (CMS, 2011). HIPAA legislation outlines stringent requirements that must be followed to protect patient privacy and confidentiality when transmitting any form of protected health information (including mental health information).

With the onset of the COVID-19 pandemic, many HIPAA policies were temporarily revised or suspended. On March 17th, 2020 the Office for Civil Rights (OCR) at the U.S. Department of Health and Human Services (HHS) declared that it would utilize its enforcement discretion to ease penalties for HIPAA violations of privacy and security during the COVID-19 public health crisis. The OCR also relaxed its breach notification rules for health care providers using technology to communicate with patients during the COVID-19 pandemic (hhs.gov, 2020).

Ideally, of course, psychotherapy sessions should be conducted in a private setting with minimal distractions and interruptions. However, the realities of the COVID-19 pandemic have, in many cases, rendered this ideal impossible to achieve. For example, in the early stages of the pandemic when entities such as businesses, schools, and mental health clinics were in lockdown, both clients and clinicians often found themselves connecting for telehealth sessions in environments that were anything but private. Many clients and clinicians were simultaneously attempting to care for elders or young children and/or facilitating at-home learning for their school-age children. Such contexts posed major challenges for clinicians and clients alike. The U.S. Department of Health and Human Services (DHHS) formally recognized that it might not be feasible to adhere to all usual HIPAA standards during COVID-19, but indicated that health care providers should continue to implement HIPAA safeguards to protect patient health information. When a private setting was not available, DHHS suggested precautions such as lowered voices and refraining from using speakerphone.

Members of the general public use myriad online platforms for personal communication purposes (e.g., Instagram, Facebook, Snapchat, Skype, etc.). However, these social media platforms are considered public-facing, and are not sufficiently secure for confidential medical and mental health conversations with patients. HIPAA mandates that any online communication with patients/clients must be conducted only via secure, HIPAA-compliant platforms designed expressly for telehealth purposes. Examples of such platforms include, but are not limited to Zoom for Healthcare, doxy.me, Microsoft Teams (with specific security controls enabled), and Spruce Healthcare Messenger (hippajournal.com. n.d.). Secure telehealth platforms have proliferated under the increased demands posed by the COVID-19 pandemic.

The Importance of Visual Cues in Psychotherapy.

Visual cues such as eye contact, body language, and facial expression provide clinicians with invaluable clues about the client's current affect, how the client is reacting to discussion of a difficult situation, and whether the client is displaying symptoms of psychopathology. When conducting therapy online, it is sometimes more difficult to detect these subtle facial expressions and nonverbal cues due to deficient image quality or the fact that only the client's face is visible (in contrast to visibility of the whole person as would be the case during in-person sessions). An additional limitation is that some of the experiential approaches used during in-person sessions are not feasible in the online environment (Alleman, 2002). Clients may experience parallel concerns because they also rely heavily on visual cues such as therapist eye contact, body language, and facial expression as signals of attentive listening and empathy on the part of the clinician. (M. K. Crothers, personal communication, June 11, 2020).

Compassion Satisfaction among Mental Health Clinicians

Stamm (2010) describes compassion satisfaction as “the pleasure one derives from being able to conduct one's professional work well, and feeling satisfied after doing so” (p. 12). People who have high compassion satisfaction feel “successful and invigorated by their work and believe they can make a difference” (Stamm, 2010, p. 21).

Senreich and colleagues (2019) found that the likelihood of compassion satisfaction increases when clinicians have a manageable caseload, believe their training has adequately prepared them for their work responsibilities, and feel their work is valued. The first two of these factors have been impacted significantly by the COVID-19 pandemic. Clinicians' caseloads have risen sharply due to the dramatic increase in the number of people seeking mental health care in response to the anxiety and isolation ensuing from COVID-19. In terms of clinicians' perceptions that their training has adequately prepared them for their work, the pandemic may have adversely affected compassion satisfaction because the majority of mental health professionals had no experience with Internet-based therapy prior to the pandemic. Further, to avoid prolonged disruptions in treatment at a time when client stress was particularly intense, the transition to online service-delivery had to be accomplished very rapidly. There was virtually no advance warning and very little time to obtain training and master the technology required for online therapy, thereby thrusting clinicians abruptly into a work environment for which they felt inadequately prepared. It is therefore plausible to imagine that clinicians' compassion satisfaction may have declined during the pandemic.

Burnout among Mental Health Clinicians

Maslach (1982) defined burnout as "a syndrome of emotional exhaustion, depersonalization, and reduced personal accomplishment" (p. 3). Other researchers have further developed the description of burnout, reporting that it tends to be characterized psychological distress, feelings of dissatisfaction, impaired interpersonal functioning, cynicism, physiological symptoms, and emotional numbing (Fothergill et al., 2004). Stamm (2010) reported that people who experience burnout express feeling "unhappy, insensitive, and disconnected regarding their professional work" (p. 21).

Factors that increase the likelihood of burnout for mental health clinicians include limited time to meet deadlines or perform duties, the psychological weight of responding to the emotional needs of clients, and peripheral administrative responsibilities (Dillenburger, 2004). Previous research has shown that heavy workloads and pressure to meet productivity targets (as measured by billable client hours per week) contribute to work-related stress (Collings & Murray, 1996). As soon as the COVID-19 pandemic began, mental health services were quickly overrun, with the need for treatment far exceeding the capacity of clinicians. This resulted in stress from increased workload and from having to turn away prospective clients who were in desperate need of support. In addition to other work-related and personal stressors, the sudden shift to entirely online service-delivery posed unique challenges, as many clinicians had no previous experience with telehealth. These factors likely increased levels of stress for clinicians, potentially contributing to decreases in their compassion satisfaction and increases in their risk for burnout.

Purposes of the Study

The purposes of the study reported here were twofold: First, the study investigated whether the sudden shift to online mental health service-delivery was associated with changes in clinician compassion satisfaction and risk of burnout. Second, the researchers sought to explore clinicians' subjective experiences and perceptions regarding the abrupt shift to telehealth during the COVID-19 pandemic. Specifically, the study explored clinicians' comfort/discomfort with telehealth, their perceptions of the positive and negative aspects of online therapy, and challenges

related to eye contact and their ability to detect subtle nonverbal cues during telehealth sessions.

Method

Participants

Participants were recruited from four mental health clinics in a circumscribed midwestern region. The recruitment process yielded 26 fully completed surveys. All participants were direct-service mental health or AODA (Alcohol and Other Drug Abuse) clinicians. Experience levels ranged from participants who were completing pre-credential internships, to seasoned clinicians with more than 20 years of professional experience.

Measures

To measure compassion satisfaction and burnout, the researchers employed a subset of items from the Professional Quality of Life Scale (ProQOL) (Stamm, 2010). The ProQOL measures the positive and negative aspects one may experience in a helping profession. It is widely used to measure the quality-of-life experiences of mental health therapists, medical health professionals, and social service employees. Selected items from the ProQOL were utilized to compare counselors' recollections of their compassion satisfaction and burnout levels before the pandemic began, with their levels of the same two variables at the time data were collected, one year into the pandemic.

The ProQOL includes three, 10-item subscales (Compassion Satisfaction, Burnout, and Secondary Traumatic Stress), each rated on a 5-point scale (1 = never; 5 = very often). Because this study did not include exploration of secondary traumatization, only the items from the Compassion Satisfaction and Burnout subscales were utilized. Subscale scores on the ProQOL are computed by summing the ratings for the items loading on each factor. For both subscales, scores ≤ 22 are considered low; scores from 23-41 are considered to fall in the medium range; and scores ≥ 42 are considered high (Stamm, 2010). The ProQOL has shown good internal consistency for both subscales, with the following Cronbach's *alpha* levels: Compassion Satisfaction $\alpha = .88$; Burnout $\alpha = .75$ (Geoffrion et al., 2019). One aim of the study was to determine whether clinicians' Compassion Satisfaction and levels of Burnout had changed across the first year of the COVID-19 pandemic. To achieve this aim, participants were asked to give two separate ratings for each ProQOL item – one retrospective rating reflecting how they were feeling when the pandemic first began and they were first starting to use telehealth, and a second rating reflecting their current feelings at the time of data collection, almost precisely one year into the pandemic.

In addition, the researchers developed a novel scale to measure the effects of the sudden, forced shift of mental health service delivery to the online environment as necessitated by the COVID-19 pandemic. This researcher-generated measure was composed of 30 items – some rated on a Likert-type scale and others worded as open-ended questions with free-response comment fields. The measure included questions about participants' perceptions of the positive and negative aspects of online therapy, their comfort (or discomfort) with telehealth, and their perceptions of their ability to establish eye contact effectively and to detect subtle nonverbal client cues when conducting psychotherapy in the online environment.

Items from the ProQOL and the novel researcher-designed measure were combined into a single Qualtrics survey. The survey design employed the “skip logic” function so that participants were asked to respond only to those items that were directly related to their specific work experiences. Basic demographic information (e.g., age, years of professional experience, and type of credential) was also gathered, but was not ultimately used in the analyses due to small sample size and potential concerns about anonymity.

Procedure

The first step in recruitment entailed contacting (by phone) the directors of four mental health clinics. The researchers expressed the desire to recruit clinical staff members to participate in an online survey exploring clinicians’ experiences with delivering client services via telehealth during the COVID-19 pandemic. The researchers provided a general description of the study procedures, offered opportunities for questions and clarification, and requested permission to contact clinical staff members to invite their participation. If the director gave permission to recruit, a list of staff names and email addresses was requested.

Each prospective participant received an individualized email invitation (and two reminder emails, at one-week intervals). The initial email included a brief description of the purpose and nature of the study and invited them to participate. Those interested in participating were directed (via a link embedded in the email) to more detailed informed consent information, which was followed by a yes/no question asking whether the potential participant understood the information and gave their informed consent to proceed with participation. Those who answered “No” to this question were thanked for their consideration and exited out of Qualtrics. Those who answered “Yes” to this question were directed automatically to the survey items. The “*anonymize response*” feature in Qualtrics was employed to ensure that no personal information about the participants was recorded. Upon completion of the survey, participants were thanked, provided with contact information for the researchers, and given the opportunity to request a summary of the findings of the study, if desired.

Results

Descriptive statistics showed that clinicians experienced decreases in compassion satisfaction between the start of the pandemic and the time of data collection one year later. Specifically, the mean of the clinicians’ retrospective ratings of their compassion satisfaction just prior to the beginning of the pandemic was 43.65, as compared to a mean of 40.50 one year into the pandemic. A paired samples t-test showed that this change in compassion satisfaction was statistically significant ($p < .001$). Cohen’s D indicated a relatively large effect size of 0.756. This decrease in compassion satisfaction is presented graphically in Figure 1, below.



Figure 1. *Clinician Compassion Satisfaction at Outset of COVID-19 Pandemic and One Year Later*

Descriptive statistics showed that clinicians experienced increased symptoms of burnout increased between the start of the pandemic ($M = 18.73$) and one year later ($M = 21.96$). A paired samples t-test revealed that this increase in burnout symptoms was statistically significant ($p < .001$). Cohen’s D indicated a relatively large effect size of 0.756. This increase in symptoms of burnout is represented graphically in the figure below.

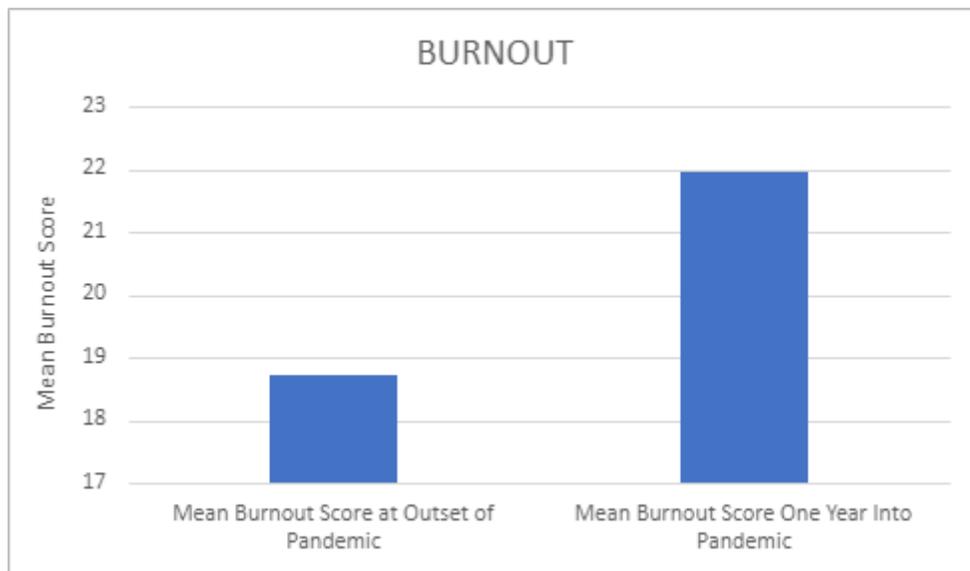


Figure 2. *Clinician Burnout at Outset of COVID-19 Pandemic and One Year Later*

Participant responses to the question about their comfort with using telehealth technology at the start of the pandemic versus one year later indicated that, in general, their comfort had increased markedly. Specifically, at the outset of the pandemic approximately 28% of

clinicians were somewhat or very comfortable using telehealth, as compared to 79% one year later. This corresponded with a decrease in the percentage of clinicians reporting some degree of discomfort, from 38% initially to only 3% one year later. These findings are represented graphically in Figure 3, below.

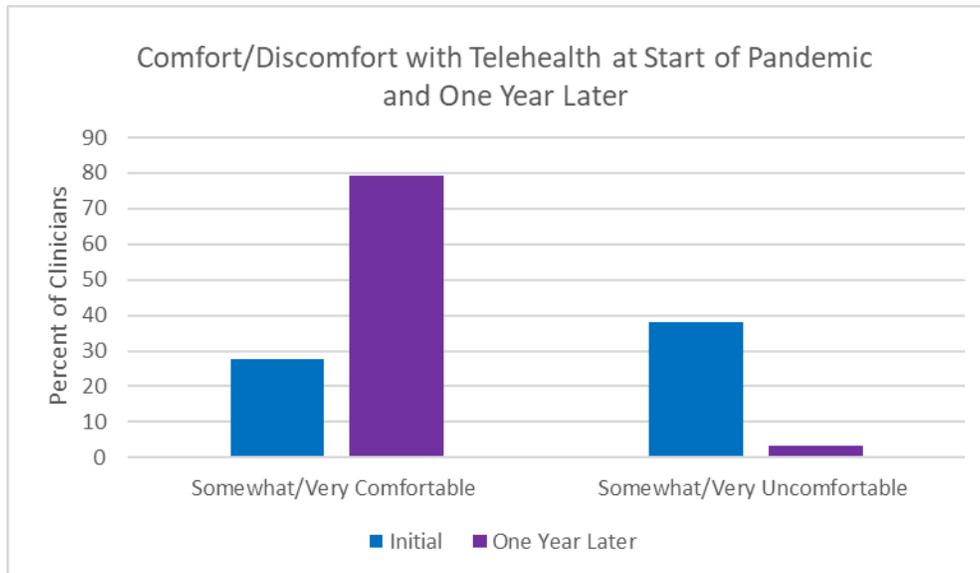


Figure 3. *Clinician Comfort with Telehealth Technology at Outset of Pandemic and One Year Later*

At the time of data collection (almost precisely one year after the start of the pandemic), only a very small proportion of clinicians (3%) had resumed fully in-person service delivery; 76% were still conducting sessions exclusively via telehealth, and 21% were still using telehealth with some clients, but had returned to in-person service delivery with others. Slightly more than half (54%) of the clinicians reported that they found conducting virtual sessions more tiring than in person sessions; however, nearly half (45%) reported enjoying interacting with clients via telehealth more enjoyable than they had anticipated would be the case.

An open-ended question asking participants to describe either positive or negative aspects of conducting online therapy yielded some interesting responses. In terms of negative aspects, some clinicians reported experiencing difficulty with eyestrain. On the positive side, clinicians noted that telehealth service delivery increases access to treatment for some clients (e.g., those who are geographically distant, or have limited mobility, or experience anxiety about driving in inclement weather). Approximately two-thirds of the clinicians reported having clients who were reluctant to meet with them over video telehealth, and 90% of those clinicians worked with video-reluctant clients via telephone. Sixty-nine percent of clinicians reported that some of their clients were unable to meet with them via video telehealth because they did not have Internet service and/or did not have an electronic device through which to connect.

The researchers were interested in exploring challenges relating to eye contact when conducting telehealth sessions. One of the most important and effective ways to communicate empathy and undivided attention is for the clinician to make direct eye contact with their client. In order

for the client to subjectively experience this type of eye contact during telehealth sessions, the clinician must look directly into the camera. However, doing so means that the clinician cannot see the subtle nonverbal facial signals that are invaluable in psychotherapy (e.g., frowning of the brow, tears welling up, etc.) It is impossible for the clinician to simultaneously look into the camera and detect these subtle cues. Participants indicated a moderate degree of initial worry about this challenge, but indicated that it interfered only slightly with their ability to communicate empathy and attentiveness successfully, and to detect subtle nonverbal cues.

Limitations

The limitations of this exploratory study include its small sample size, circumscribed geographic region, and the limited variety of mental health settings from which the sample was recruited. Further, the novel survey items developed by the researchers have not yet been subjected to rigorous psychometric validation. The design of the study does not explore the many additional variables that contribute to compassion satisfaction and burnout. Future studies will be strengthened by addressing these limitations.

Discussions

As expected, clinicians reported decreases in compassion satisfaction and increases in symptoms of burnout across the course of the first year of the COVID-19 pandemic. Although the present study focused on clinicians' experiences with the sudden shift to telehealth, and that shift quite likely had an impact on compassion satisfaction and burnout, it is essential to emphasize that the findings reported above do not support causal inferences. Myriad factors other than the shift to telehealth almost certainly influenced the decrease in clinician compassion satisfaction and the increase in clinician burnout symptoms.

Although more than a third of clinicians reported initial discomfort with the prospect of shifting to telehealth service delivery, only three participants reported any lingering discomfort one year into the pandemic. Further, although the clinicians experienced more fatigue and eye strain in the online therapy environment than when conducting in-person sessions, many of those who were initially reluctant about telehealth found it more enjoyable than expected. Taken together, these findings reflect remarkable clinician adaptation under challenging circumstances.

Caveat

While online service delivery increases convenience and the accessibility of mental health treatment for *some* clients, it is essential to recognize that it can potentially introduce barriers for others, sometimes rendering treatment completely inaccessible. For example, many remote geographical areas lack reliable Internet service and people living in poverty may not have equitable access to electronic devices. These barriers may reduce access to mental health care for some of the very individuals who most urgently need it. It is imperative that we not allow enthusiasm over the convenience of telehealth technology to overshadow the inherent access disparities. Future research should focus on identifying these and other potential disparities and

barriers, as well as developing and measuring the efficacy of programs designed to address them.

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“Before you make a dream come true, you must first have one.” - **Ronald. E McNair**