Hendrick Hudson High School

SCIENCE

RESEARCH

May 2019

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SCIENCE RESEARCH

May 2019

Congratulations to a wonderful group of students, smart, witty and harboring a great sense of humor.

You all teach me every day and for this I am grateful. You all have grown up so much in the past few years.

Thea Barbelet has established herself as a team leader, providing help to all of the students. She also won first place for her poster in the Animal Science category at JSHS. She place fourth at WESEF.

Hailey Kissner has been organizing our parties all year. She placed third at the Tricounty Fair for her project in social and clinical psychology. She has been recognized by the Haskins Laboratories, affiliated with Yale University and the University of Connecticut for her outstanding work. Hailey will be presenting her work to several schools working with dyslexic students in the tristate area.

Buu-Hac Nguyen won second place in Cellular and Molecular Biology at WESEF for her work on nanobodies and the dopamine transporter.

Junior **James Reilly** placed fourth in the same category for his work on probiotics and their influences on the dopamine transporter. Both James and Buu-Hac worked with **Dr. Matthias Quick** at Columbia Presbyterian in the Department of Psychiatry and the Center for Molecular Recognition.

Junior **Paul Williams** placed third at NYSSEF (New York State Science and Engineering Fair) in medicine and health category for his work on nanoparticles at Memorial Sloan Kettering Institute with Dr. Zvi Yaari.

All seniors students produced high quality papers, such that they were able to enter the Science Talent Search competition.

My outreach work continues with the Pine Ridge Girls School in South Dakota, to develop a research program with their students and foster a partnership. We delivered supplies last summer and I will be returning to train science teachers. Special thanks to Mr. and Mrs. Chung and Mr. James Mackin for their generous donations. We hope to have a regular collaboration by next year. I am very grateful to all who have been involved in creating and supporting this collaboration. Multicultural exchanges and collaborative work promote different point of views and foster resilience, grit and team work skills

Effects of Modification of Floral Guides on Apis mellifera and Exposure to Neonicotinoids. by Thea Barbelet, STS Paper Excerpt, Senior

Abstract

Pollinators face a multitude of threats, including parasites, habitat loss, and pesticide exposure. The effects of these perils are clearly seen in the hives of honey bees, where populations are decreasing and behavior is changing. This is worrisome because honey bees are a keystone species and if they go extinct, two-thirds of our food sources will run out and entire ecosystems will fail. Since honey bees are attracted to the ultraviolet markings on flowers that are hidden to human eyes, removal of these markings will cause honey bees to be uninterested in the flowers. Additionally, all honey samples may be contaminated with pesticides, even if they were not applied directly to the flowers, because pesticides easily spread throughout the environment. To test this, I purchased tickseed bushes with natural ultraviolet markings. One-third of the bushes were unaltered and acted as the control group, another third was covered with lotion sunscreen, and the last third was covered with spray sunscreen; both effectively blocked the ultraviolet markings. I also acquired honey samples from local apiaries and sent them to Intertek's labs to be analyzed for the presence of pesticides. Once data was collected, it was clear that honey bees had a significant decrease of interest in flowers treated with either sunscreen. The spray sunscreen proved the most effective in achieving disinterest, as that group had the lowest number of landings. Contrary to expectations, all of the honey samples tested negative for pesticides. Due to the small scale of this study, the effects of rainy weather, location of wildflowers, time between pesticide application and pollination, and the type of pesticides applied are unknown. So, flowers treated with fluorescent pesticides may be more attractive to honey bees; thus, increasing the likelihood of honey bees visiting treated flowers and therefore becoming contaminated with pesticides and bringing those contaminants back to the hive. This study shows the importance of ultraviolet markings on flowers and how easily they can be disrupted.

Introduction

Bees and flowers provide the most blatant example of coevolution. Prehistoric amber dates the first existence of pollinators at over 100 million years ago (Murillo-Barroso et al., 2018; Peñalver et al., n.d.), and a sample from Cretaceous New Jersey holds a 96 million year old member of the Apoidea superfamily (Michener & Grimaldi, 1988). Similarly, phylogenetic evidence shows that Mesangiospermae, or early core Angiosperms, began to diversify at least 146 million years ago (Doyle, 2012).

Before Mesangiospermae, no plants possessed petals, and would instead release their pollen into the wind, a relatively ineffective system. If pollen did not reach the sex parts of a Mesangiospermae of the same species and successfully pollinate it, the species could eventually die out. Through evolution, plants with nectar and white petals were selected to provide a better dissemination of pollen, evidenced by current day Magnolia acuminata (Doyle, 2012). The high sucrose content of nectar enticed early insects and the white petals created distinction from the rest of the environment. When insects fed on these Mesangiospermae, pollen stuck to their bodies and was inadvertently carried from plant to plant, ensuring the species' survival and creating a new method of pollination.

Despite the passing of millions of years, insect pollination remains a prevalent method of pollination for today's Angiosperms (Cappellari, Schaefer, & Davis, 2013). Now, both Angiosperms and pollinating insects are found in a wide variety of species, each with different shapes, sizes, and colors. Additionally, Angiosperms now utilize long range and close range floral guides to draw in pollinators. Long range floral guides are the scent, symmetry, shape, or color of Angiosperms while close range floral guides are the ultraviolet markings of only some Angiosperms (Horth, Campbell, & Bray, 2014). Effective ultraviolet markings can appear as a dark centered blotch, a bull's-eye, or as lines acting as a landing strip (Orbán & Plowright, 2014).

The majority of insects have compound eyes and bichromatic vision. Yet, some - oftentimes pollinators - have trichromatic vision which enables them to perceive nearly all the visible light that humans can see, as well as ultraviolet light. Humans can see electromagnetic wavelengths from 400 to 800 nanometers while trichromat insects see from 300 to 650 nanometers. This enables them to see ultraviolet light, but have difficulty registering hues of red, orange, and yellow (Briscoe & Chittka, 2001). Therefore, the ultraviolet close range guides of angiosperms are visible to Western honey bees, Apis mellifera (Hempel De Ibarra, Vorobyev, & Menzel, 2014). The same goes for Papilionoidea, or butterflies, with 15 photoreceptors that allow them to detect red, orange, and yellow as well. Papilionoidea can distinguish and visit flowers that A. mellifera might miss (Frentiu et al.,2007) .

A. mellifera reign as the most effective pollinators, while more niche needs are

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There are many threats to pollinators, especially *A. mellifera*. *Varroa destructor* mites, *Nosema apis* gut parasites, habitat destruction, and many forms of pesticides play a role in the wide-scale deaths of *A. mellifera* (Vanengelsdorp et al., 2011).

In the United States, herbicides and insecticides are the most commonly used pesticides. Roughly 85% of herbicides are used in the agriculture industry, while 50% of insecticides are used by consumers at home (EPA US et al., 2008). Thousands of pounds of pesticides are applied yearly in the US, with atrazine, organophosphates, and newly-developed neonicotinoids being the most common (Alavanja, 2009).

Atrazine is a pre-emergence herbicide, destroying weeds before they can fully grow. Therefore it poses no threat to honey bees (Cornell University, Michigan State University, Oregon State University, 1993). Organophosphates work by disrupting signals sent between nerves and muscles, with sarin being an extreme example of their toxicity (EPA & Pesticide Programs, n.d.). Neonicotinoids function similarly to organophosphates, attacking the nervous system and eventually causing disorientation and paralysis (Iwasa et al., 2004). Organophosphates and neonicotinoids typically do not kill immediately and instead alter the behavior of A. mellifera and weaken entire colonies (Mitchell et al., 2017). Organophosphates and neonicotinoids are essentially low-grade nerve agents (Connolly, 2017).

While

Fig 1: Bidens ferulifolia, or tickseed, under visible light (above) and ultraviolet light (below). Ultraviolet induced visible fluorescence photography allows humans to simulate pollinator vision. The darkened center of the floral head is an example of a close range floral guide (Rørslett, "Bidens cf. ferulifolia", 2006). Note: the common name "tickseed" is shared by more than one species, with Bidens and Coreopsis being the most biologically similar

met by other local pollinators, including different species of wasps, hoverflies, butterflies, and other eusocial and presocial bees. *A. mellifera* contribute $20 billion worth of crops to the American economy and $200 billion worldwide (Fairbrother, Purdy, Anderson, Fellk, & Bellevue, 2014). Environmentally, *A. mellifera* are a keystone species, providing invaluable services to an ecosystem. Without them, and the other pollinators that carry out any jobs *A. mellifera* leave unfulfilled, entire ecosystems could fail (Hung et al., 2018). Biodiversity losses would be staggering and the world as a whole would suffer greatly without pollinators.

designed to kill pests, pesticides often affect entire ecosystems. Professional and personal application of pesticides allows dangerous chemicals to spread, affecting surface water, groundwater, soil, turf, and the air (Aktar et al., 2009). If a farmer applies herbicide to their crops, there is little guarantee that it will not reach humans in one way or another. Many pesticides, such as thiamethoxam and bifenthrin are possible or likely human carcinogens, and fluvalinate can cause birth defects (Lozowicka et al., 2014). Pesticides pose a risk to both A. mellifera and the human population .

Many pesticides have naturally fluorescent properties. In one study, over thirty pesticides tested had excitation wavelengths at roughly 254 nm (Argauer, 1980). Benomyl, coumatetralyl, diphacinone, fuberidazole, propyl isome and quinomethionate are some notable fluorescent pesticides (Mallet et al., 1973) and appear fluorescent yellow-green under ultraviolet light (Guilbault, 1990). An excitation wavelength at 254 nm would be visible to *A. mellifera* and other trichromats (Briscoe & Chittka, 2001). Additionally, when placed in
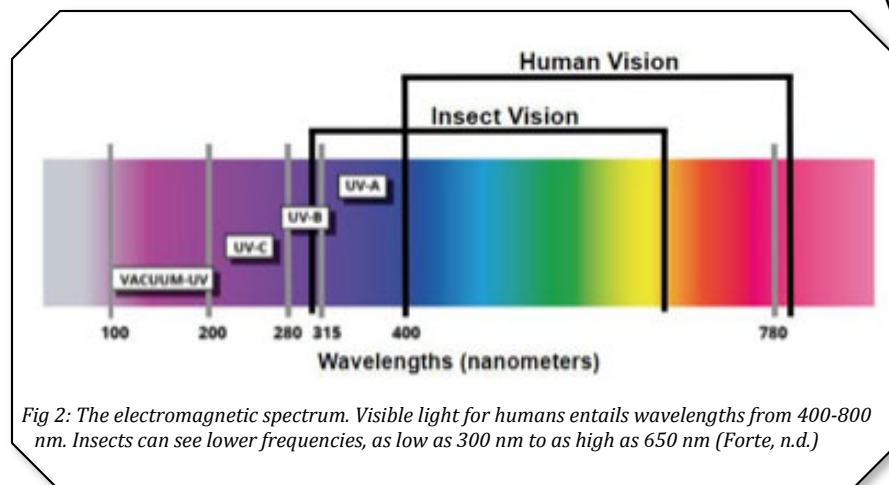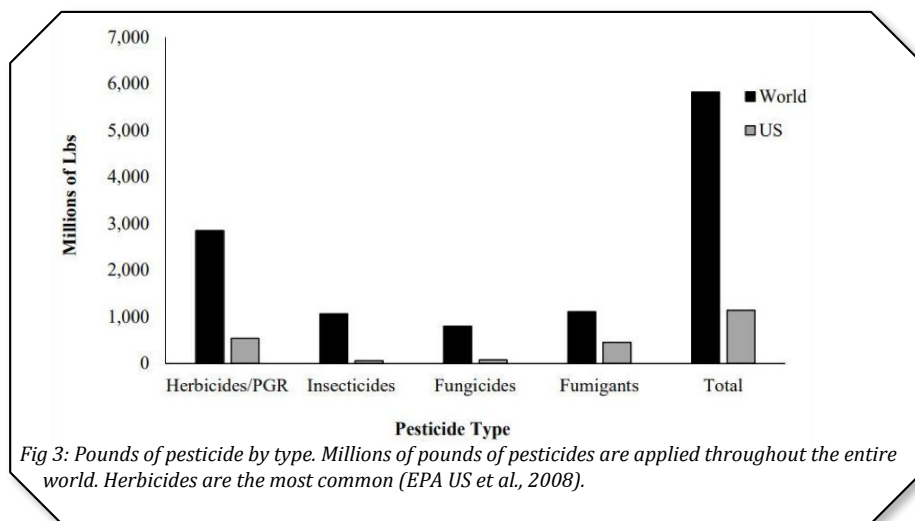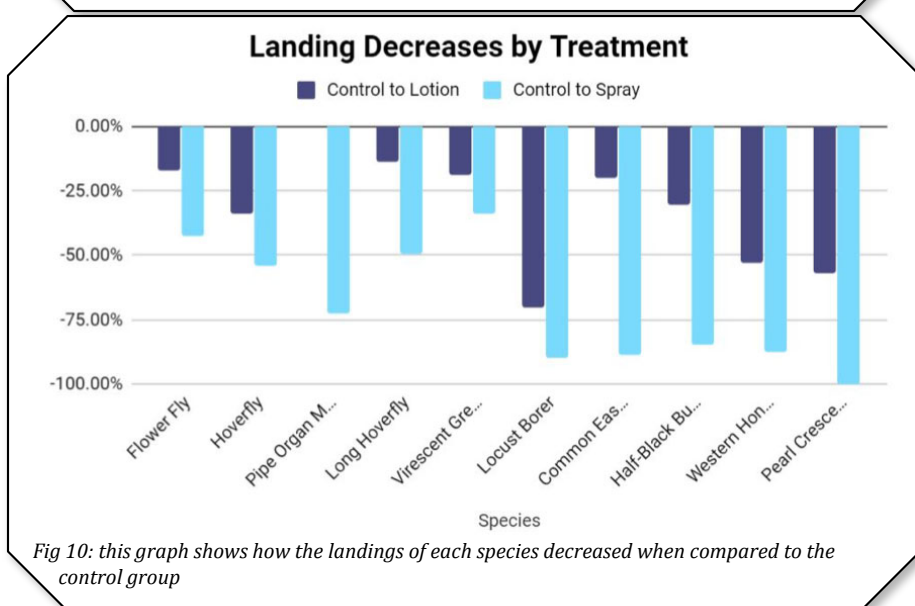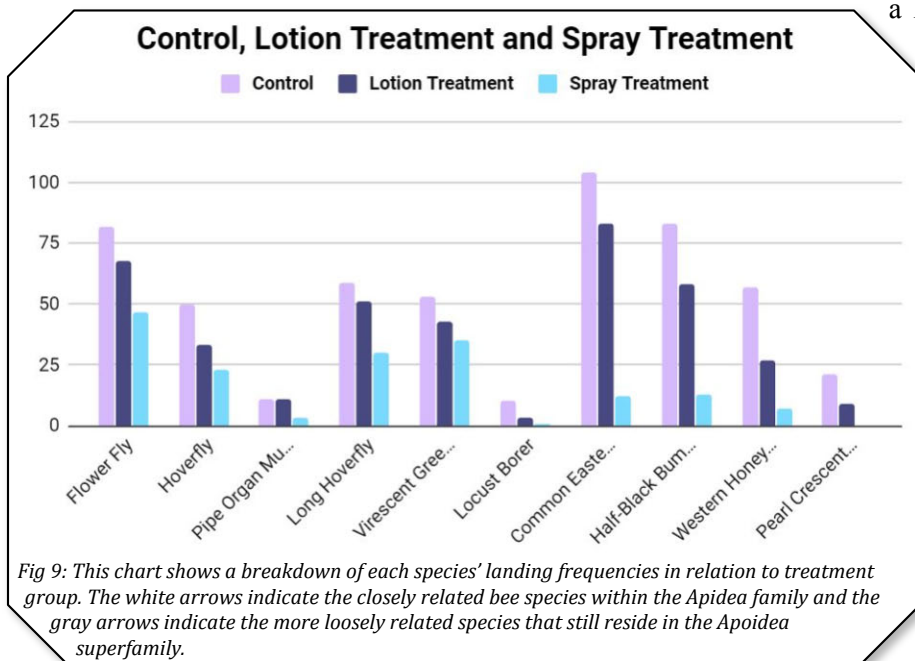

Fig 2: The electromagnetic spectrum. Visible light for humans entails wavelengths from 400-800 nm. Insects can see lower frequencies, as low as 300 nm to as high as 650 nm (Forte, n.d.)

Fig 3: Pounds of pesticide by type. Millions of pounds of pesticides are applied throughout the entire world. Herbicides are the most common (EPA US et al., 2008).

sugar solution, many neonicotinoids have shown to be attractive to *A. mellifera* and buff-tailed bumble bees, *Bombus terrestris*. Both species were shown to prefer imidacloprid or thiamethoxam laced-sugar water when presented with both contaminated and uncontaminated solutions (Kessler, et a l . ,

Fig 9: This chart shows a breakdown of each species' landing frequencies in relation to treatment group. The white arrows indicate the closely related bee species within the Apidea family and the gray arrows indicate the more loosely related species that still reside in the Apoidea superfamily.

Fig 10: this graph shows how the landings of each species decreased when compared to the control group

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2015). To pollinators, the alluring features of pesticides outweigh their danger - which may be unknown to them. .

Statement of Purpose

In the present study, I analyze both the relationship between *Apis mellifera* and *Coreopsis verticillata* and the relationship between *A. mellifera* and neonicotinoids. First, I look at pollinator landing frequencies and threadleaf tickseed when close range floral guides are modified to determine if flowers with ultraviolet markings are preferred. Then, I look at the presence of pesticides in honey from beekeepers that claim to either use or abstain from neonicotinoids. If close range ultraviolet floral guides have a significant role in attracting pollinators, then their modification may affect the frequency of pollinator visits, keeping *A. mellifera* from coming into contact with flowers treated with fluorescent pesticides. Honey bees are an important species and any information that can be gathered for their benefit is crucial.
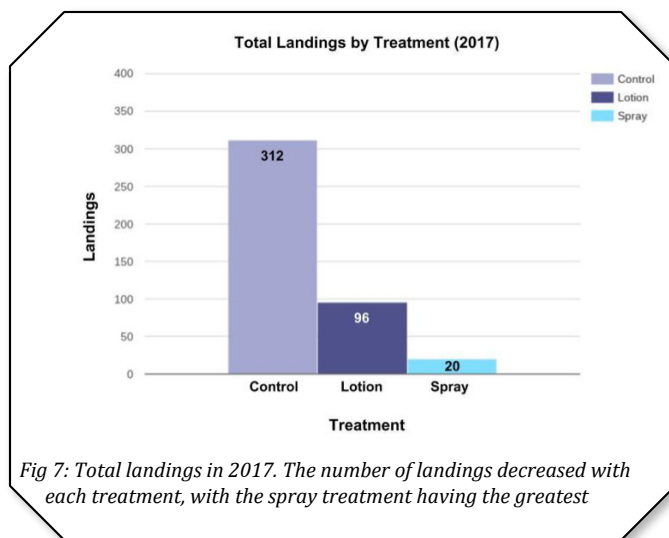
Hypotheses .

Hypothesis One - If the floral guides of the *C. verticillata* are modified, then the frequency of *A. mellifera* visits will decrease, because they rely on vision to effectively find flowers.

Hypothesis Two - Even if beekeepers claim that the foraging materials of their *A. mellifera* are not treated, honey samples will still contain pesticides, because pesticides can contaminate entire ecosystems .
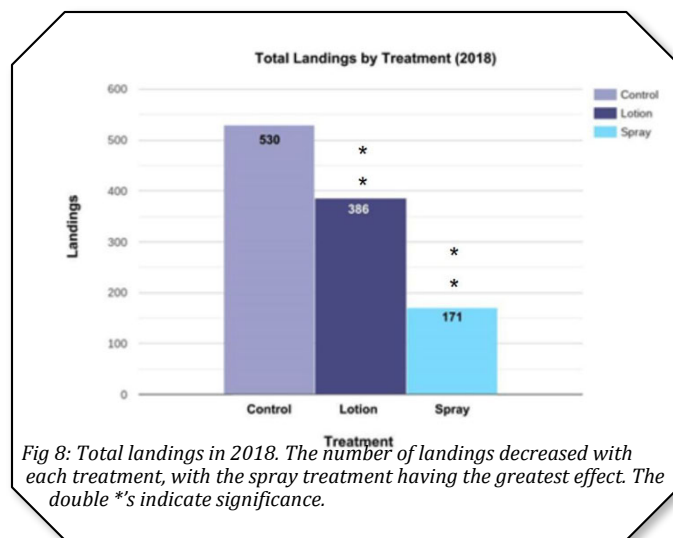
(Materials and Methods omitted)

Results

Study One - Results from the previous year's research were strengthened by this year's data collection. In 2017, 428 total landings were recorded (Fig 7), while 1,087 total landings were recorded in 2018 (Fig 8). Despite the more than doubling of total landings, the trends from both years were the same. Landings were high in the control group and decreased from the lotion treatment group to the spray treatment group.

Individual species counts (Fig 9) also decreased with each treatment. When

Fig 7: Total landings in 2017. The number of landings decreased with each treatment, with the spray treatment having the greatest

was

Fig 8: Total landings in 2018. The number of landings decreased with each treatment, with the spray treatment having the greatest effect. The double *'s indicate significance.

broken down by species, the most dramatic changes are seen in the *Apoidea* superfamily, or various bee species(Fig10).

For example in Fig 10, the *Bombus impatiens* visits, or the common eastern bumble bee, decreased 20.19% from the control group to the lotion treatment group and a striking 88.46% from the control group to the spray treatment group. *A. mellifera* had 52.63% less landings on the lotion treated flowers and 87.72% less landings on the spray treated flowers

As in Fig 11 and Fig 12, the overall 3 x 10 contingency table chi-square

Fig 11: Overall chi-square analysis.

3 x 10 Contingency Table	
Chi-Square Value	70.26739138
Degrees of Freedom	18
P-Value	0.0000000407

Fig 12: Chi-square group comparisons

	Control v Lotion	Control v Spray	Spray v Lotion
Chi-Square Value	10.09852375	60.68008504	43.26245862
Degrees of Freedom	9	9	9
P-Value	0.3425682289	0.000000000991	0.000001929173
Rows x Columns	10 x 2	10 x 2	10 x 2

analysis shows a chi-square value of 70.26739138 and a p-value inferior to .0001. This significant p-value makes chi-square group comparisons possible. These comparisons are significantly different, with each p-value being inferior to .001

Study Two – Analysis of both honey samples was provided by Intertek labs.Both honey samples did not contain pesticides. The analysis method

accredited by Intertek labs.

Discussion

In this experiment, we compared the frequency of pollinator visits after the inhibition of ultraviolet close range visual guides as well as searched for the presence of neonicotinoids in honey. Some changes were made to the study from 2017 to 2018. In 2018, Japanese beetles, *Popillia japonica*, were not considered in the landing counts. Additionally, the weather was considerably similar and favorable for all data collection days. Lastly, a likely more attractive native plant, *C. verticillata*, was used instead of the non-native species, *Tagetes patula*, used in 2017. These changes are likely responsible for the increased number of landings in 2018.

The high frequencies of hoverflies: *Syrphus ribesii* (flower fly), *syrphus torvus* (hoverfly), and *sphaerophoria script* (long hoverfly) may have been due to their attraction to the fragrant

sunscreen, as hoverflies heavily rely on their sense of smell to pollinate (Larson, Kevan, & Inouye, 2001).

The first hypothesis was supported but the second one was not. We can say that blocking the floral guides on flowers can decrease the number of pollinators that visit the modified flower. Despite pesticides, especially neonicotinoids, being well-studied contaminants, the results of this study refute the idea that untreated flora will contain traces of pesticides.

Yet, we can speculate as to why neither honey sample tested positive for neonicotinoids. Due to the small scale of this study, the effects of rainy weather, location of wildflowers, time between pesticide application and pollination, and the type of pesticides applied are unknown. Both honey samples were labelled as "wildflower honey". The wildflowers could have been relatively secluded and far away from any plants that would have been purposefully treated with neonicotinoids. The exact time of honey collection is also unknown, so pesticides could have been applied far before the honey bees' pollination or even applied afterwards. Both of these instances would result in no pesticide detection. Weather conditions are also unknown. Frequent rain may have washed away any residue that pollinators may have come into contact with. Lastly, the pesticides applied may have been pre-emergence pesticides and that were undetectable once the wildflowers bloomed and were

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Honey Sample A

Parameter	Result	Unit	Method
Imidacloprid	n.d.	µg/kg	(a)[1]
Clothianidin	n.d.	µg/kg	(a)[1]
Thiamethoxam	n.d.	µg/kg	(a)[1]
Acetamiprid	n.d.	µg/kg	(a)[1]
Thiacloprid	n.d.	µg/kg	(a)[1]
Dinotefuran	n.d.	µg/kg	(a)[1]
Nitenpyram	n.d.	µg/kg	(a)[1]

Fig 13:Analysis of honey samples A showed no detection of pesticide

Honey Sample B

Parameter	Result	Unit	Method
Imidacloprid	n.d.	µg/kg	(a)[1]
Clothianidin	n.d.	µg/kg	(a)[1]
Thiamethoxam	n.d.	µg/kg	(a)[1]
Acetamiprid	n.d.	µg/kg	(a)[1]
Thiacloprid	n.d.	µg/kg	(a)[1]
Dinotefuran	n.d.	µg/kg	(a)[1]
Nitenpyram	n.d.	µg/kg	(a)[1]

Fig 14:Analysis of honey samples B showed no detection of pesticide

pollinated.

In the future, changes would be proposed to gather more satisfactory data and address problems encountered in this study. Time and funds were limited. This study should also be repeated over a larger area in order to account for more pollinator species.

Conclusion

Pollinators are invaluable and it is important that we understand how they are exposed to pesticides and the consequences of that exposure. The revised hypothesis is as follows:

Hypothesis Two – The contamination of foraging materials of A. mellifera depends on varying external factors, including time, weather, location, and material type, even if pesticides have or have not been directly applied in the area.

(citations available upon request)

Single-Domain Antibody-Mediated Modulation of Neurotransmitter Transport by Buu-Hac Nguyen, STS Paper Excerpt, Senior

Abstract

The human dopamine transporter (hDAT) maintains dopamine homeostasis in the central nervous system. hDAT is the molecular target of therapeutic and illicit substances such as cocaine or amphetamines. These compound, like virtually all 'classical' drugs, exhibit side effects that result from unwanted interactions with other proteins in the body. One promising approach to overcome this problem is the use of small proteins that, in theory, can exclusively modulate hDAT function. In this study I analyzed the effect of single-domain antibodies (nanobodies) that were raised against a bacterial homolog of hDAT, LeuT, on the function of LeuT and hDAT. The results of this study portray how the nanobodies affect alanine uptake activity by LeuT, either by decreasing or increasing LeuT-mediated alanine uptake activity. Testing dopamine uptake by hDAT in the presence of the nanobodies revealed that the nanobody-specific modulation pattern of transport activity measured for LeuT was observed for hDAT function. The modification of function of hDAT by nanobodies that were raised against a bacterial homolog of a protein implicated in human psychiatric health and disease may lead to the successful use of highly specific small proteins as powerful agents in immunotherapy.

Introduction

The brain is the control center for behavior and emotions. Fast communication between nerve cells (neurons) in the brain along neuron extensions, or axons, is mediated by electrical impulses called action potentials, or a change in voltage across a cell membrane, that move along the axon (Figure 1). Communication between axons is achieved through a process called neurotransmission. Neurotransmission is the process by which chemical messages are transmitted from neuron to neuron – a linkage between action potentials.
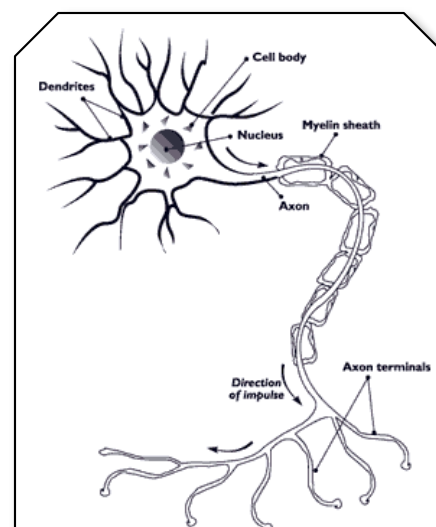

Figure 1. The following diagram portrays a neuron, where action potential occurs.
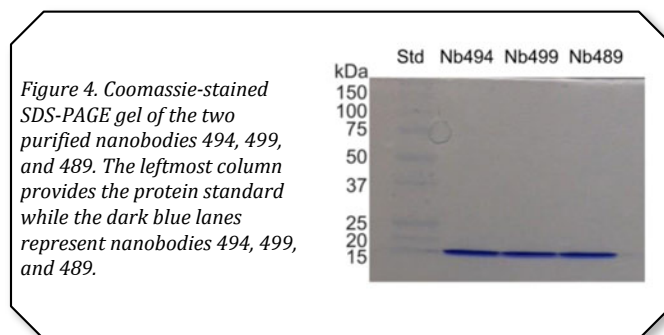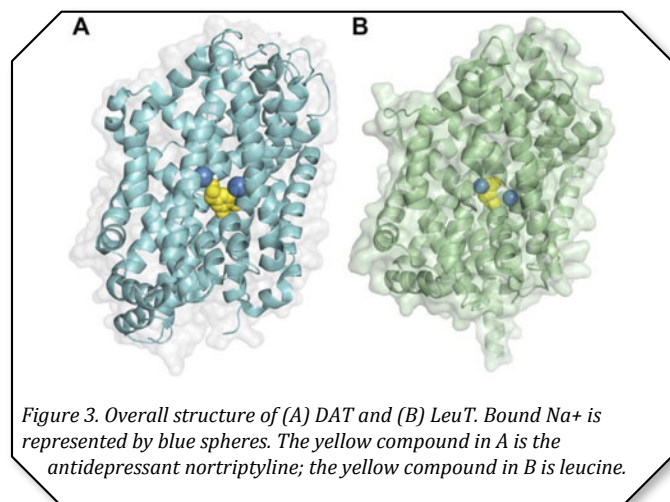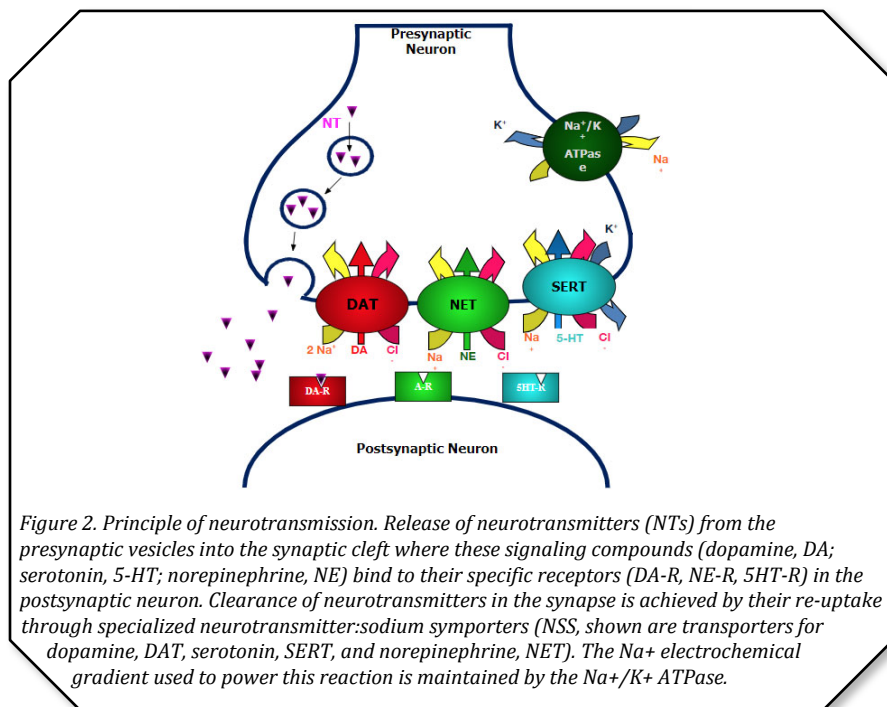
These nerve impulses are transmitted by neurotransmitters that are released from the axon terminal of a neuron and bind to the receptors in the postsynaptic membrane of another neuron. Some important neurotransmitters are the biogenic amines dopamine, serotonin, and norepinephrine. Some of the functions of these neurotransmitters include regulating the motivation-reward system, mood, social behavior, sleep, and memory. Imbalance of this process can result in harmful consequences, such as depression. Key players in the process of neurotransmission are the neurotransmitter:sodium symporters (NSS), such as the dopamine transporter (DAT), serotonin transporter (SERT), and norepinephrine transporter (NET).

Figure 2. Principle of neurotransmission. Release of neurotransmitters (NTs) from the presynaptic vesicles into the synaptic cleft where these signaling compounds (dopamine, DA; serotonin, 5-HT; norepinephrine, NE) bind to their specific receptors (DA-R, NE-R, 5HT-R) in the postsynaptic neuron. Clearance of neurotransmitters in the synapse is achieved by their re-uptake through specialized neurotransmitter:sodium symporters (NSS, shown are transporters for dopamine, DAT, serotonin, SERT, and norepinephrine, NET). The Na+ electrochemical gradient used to power this reaction is maintained by the Na+/K+ ATPase.

NSS play a central role in signal transduction by terminating neurotransmission, that is, these transporters clear the synapse of free neurotransmitters through a reuptake mechanism according to which the released neurotransmitters are transported back into the presynaptic neuron (Figure 2). This is important in regard to maintaining homeostasis that regulates mood and behavior. The NSS use the energy of the sodium gradient across the neuronal membrane to energize the transport of neurotransmitters. For example, DAT functions through cotransport of sodium ions (Na+) and dopamine, thereby pumping dopamine out of the synaptic cleft and into the neuron interior. This is important as dopamine plays a critical role in movement, reward, behavior, and more. The DAT allows the conclusion of dopamine neurotransmission and therefore maintains dopamine homeostasis in the central nervous system.
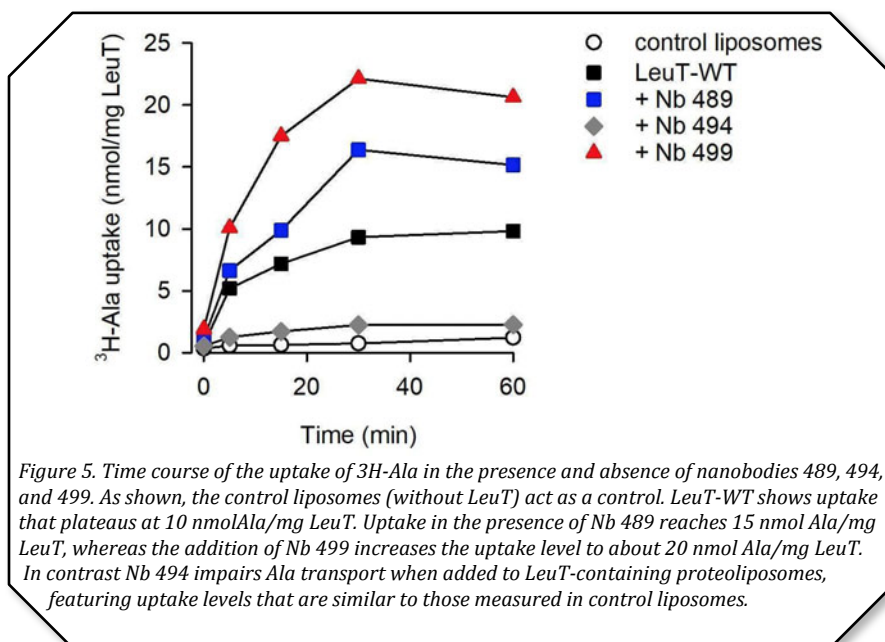
NSS have gained medical interest as they are the target of psychoactive compounds that are used in therapeutic applications (e.g., SSRIs) as well as in abuse (e.g., cocaine). Selective serotonin reuptake inhibitors (SSRIs) are a class of drugs that are typically used as antidepressants in treating depression or anxiety while cocaine is often used as a recreational drug. Some side effects of these drugs are nausea, insomnia, agitation, increased heart rate, and potentially seizures. Since NSS malfunction is directly implicated in psychiatric diseases, several psychoactive drugs specifically interact with these transport proteins (Moncrieff et al., 2013). However, to circumvent unwanted side effects associated with drug-based therapies, it may be desirable to develop molecules that exhibit high specificity and efficacy for their NSS target without eliciting these unwanted side-effects. A previous study has shown the application of 5 whole antibodies in the dopamine transporter (Ciliax et al., 1995). The advantages of using whole antibodies in this study was their specificity to DAT in rats and human striatal membranes, making possible, in theory, that antibodies could be used in immunotherapies. However, whereas classical antibodies oftentimes exhibit cross-reactivity with different protein targets, the recent development involving single-domain Antibodies (sdAb), also known as nanobodies, that exhibit affinities for their target in the nanomolar range (Pardon et al., 2014) may overcome the problem with cross-reactivity. Single-domain Antibodies are antibody fragments derived from

Figure 3. Overall structure of (A) DAT and (B) LeuT. Bound Na+ is represented by blue spheres. The yellow compound in A is the antidepressant nortriptyline; the yellow compound in B is leucine.

Figure 4. Coomassie-stained SDS-PAGE gel of the two purified nanobodies 494, 499, and 489. The leftmost column provides the protein standard while the dark blue lanes represent nanobodies 494, 499, and 489.

camelids and comprise of a heavy chain homodimer rather than of light chains, though remain fully functional (Saerens et al., 2010). One of the advantages of single-domain Antibodies is they can be produced from libraries in E. coli (Rasmussen et al., 2011) allowing the large production of single-domain Antibodies. Additionally, studies have shown the antigen-binding portion of the heavy-chain antibodies has a greater tendency to interact with parts of the target not easily recognized by conventional antibodies because they are smaller in size, highly soluble, distinctly specific, remain stable under denaturing agents, and show high affinities for their target (Cortez-Retamozo et al., 2004).

These superior characteristics allow the testing of nanobodies on a target involved in human health and disease, portraying potential use on the dopamine transporter, which allows the conclusion of dopamine neurotransmission and therefore maintains dopamine homeostasis in the

Figure 5. Time course of the uptake of 3H-Ala in the presence and absence of nanobodies 489, 494, and 499. As shown, the control liposomes (without LeuT) act as a control. LeuT-WT shows uptake that plateaus at 10 nmolAla/mg LeuT. Uptake in the presence of Nb 489 reaches 15 nmol Ala/mg LeuT, whereas the addition of Nb 499 increases the uptake level to about 20 nmol Ala/mg LeuT. In contrast Nb 494 impairs Ala transport when added to LeuT-containing proteoliposomes, featuring uptake levels that are similar to those measured in control liposomes.

Mentor:

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central nervous system. The nanobodies would thus be used as an activity-modifying drug-like compound, without the unwanted side effects associated with 'classical' drug-based therapies. This study first uses the leucine transporter (LeuT) to raise nanobodies against LeuT. Due to the similar structure between the dopamine transporter and the leucine transporter, it is predicted these nanobodies also bind to DAT. The second part of the study focuses directly on the DAT, directing nanobodies against the DAT to determine if nanobodies can be used as immunotherapy agents.

Statement of Purpose

Nanobodies have emerged as powerful molecular tools to interact with membrane proteins with almost unparalleled specificity and affinity. Using nanobodies that were raised against the bacterial homolog of neurotransmitter: sodium symporters (NSS), LeuT, the goal of this study is to test my hypothesis that due to the structural conservation of NSS members, the LeuT-based nanobodies interact with the dopamine transporter, a NSS member that is implicated in psychiatric conditions and drug abuse.

(Materials and Methods omitted)

Results

I started my project with the expression and purification of the nanobodies. The Coomassie-stained SDS-PAGE gel of the purified nanobodies 494, 499, and 489 were subjected to size-exclusion chromatography and shows how the proteins are found in a highly pure form (Figure 4). Thus, following the expression/purification protocol, I was able to purify the nanobodies to apparent homogeneity.

The time course of 3H-Ala uptake was tested in proteoliposomes that contain LeuT (Figure 5). Uptake was performed in the absence or presence of Nb494, Nb499, or Nb489. With Nb494, uptake of 3H-Ala was almost completely inhibited, whereas, in stark contrast, uptake in the presence of Nb499 was about double of that observed for LeuT in the absence of a Nb. Likewise, uptake in the presence of Nb489 also showed an increase relative to the uptake observed for LeuT in the absence of a Nb. This denotes the nanobodies used in this study alter the function of LeuT which presents ideal results to test the nanobodies against DAT, as LeuT acts as a reliable template to determine DAT structure-function predictions.

DAT Characterization

Figure 6 shows the uptake of DA in the presence and absence of nanobodies 489, 494, and 499. DA uptake in control groups across all treatments reached approximately 5 pmol/5 x 105 cells. Referencing the bright red columns, the uptake in no treatment group (sans
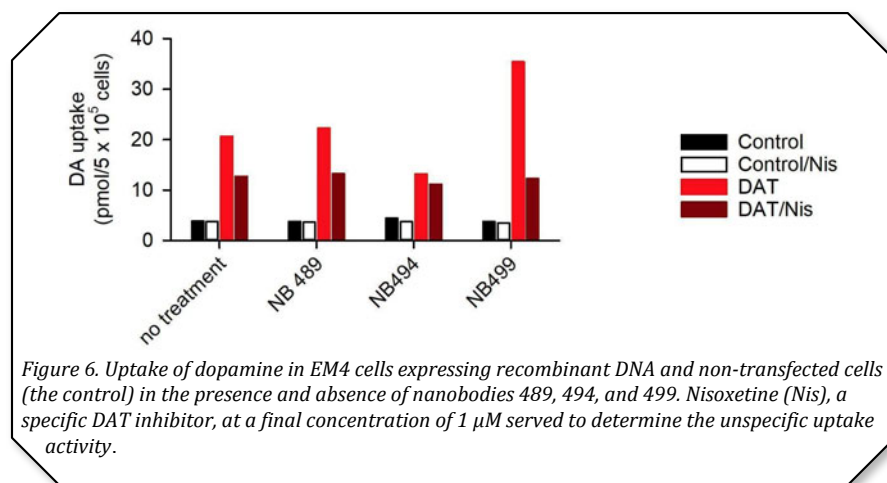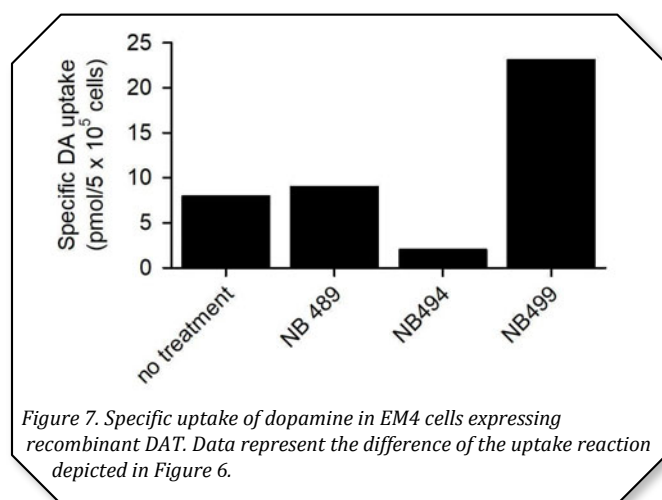
Figure 6. Uptake of dopamine in EM4 cells expressing recombinant DNA and non-transfected cells (the control) in the presence and absence of nanobodies 489, 494, and 499. Nisoxetine (Nis), a specific DAT inhibitor, at a final concentration of 1 µM served to determine the unspecific uptake activity.

nanobodies) reaches 20 pmol/5 x 105 cells. Uptake in the presence of Nb489 shows a slight increase relative to the uptake in no treatment. Next, uptake in the presence of Nb494 reaches approximately 12 pmol/5 x 105 cells, which was less DA uptake than the uptake in no treatment, inhibiting transporter function. On the other hand, DA uptake in the presence of Nb499 reaches almost 40 pmol/5 x 105 cells, almost double the uptake than no treatment, stimulating DAT. Similar to nanobodies directed against LeuT, the nanobodies directed against DAT altered the function of the transporter.

Figure 7 displays the specific DAT uptake activity. This was determined by subtracting the background determined with nisoxetine, a drug that blocks DAT function, from the total uptake activity (measured in the absence of nisoxetine). This calculation was performed to show if there is any DAT-specific gain or loss of function due to the test condition.

Figure 7. Specific uptake of dopamine in EM4 cells expressing recombinant DAT. Data represent the difference of the uptake reaction depicted in Figure 6.

After taking account of the difference between total uptake and the background, it is clear Nb489 slightly enhanced the function of DAT, Nb 499 dramatically enhanced the function, and Nb 494 inhibited the transporter's function. Therefore, there was DAT-specific gain and loss of function due to the presence of nanobodies 489, 494, and 499.

Discussion

Compared to whole antibodies, nanobodies are significantly smaller, which alters the biodistribution of the single-domain antibodies and improves access to the target. Other advantages of nanobodies are the ability to block certain proteins for biochemical and crystallographic studies, can be screened for specifically desired conformation, and can be produced from libraries in E. coli (Cortez-Retamozo et al., 2004). Nanobodies also show high affinities for their target and remain stable under denaturing agents (Pia et al., 2015); with these superior characteristics, nanobodies portray potential use as tools in immunotherapy.

In this study, nanobodies in a highly pure form were first directed against a homolog of hDAT, LeuT, as a reliable template to determine DAT structure-function predictions. Referencing the graph displaying the uptake of alanine, the presence of Nb499 reaches 20 nmol/mg LeuT which portrays a double alanine uptake relative to the uptake observed for LeuT in the absence of a Nb. The presence of Nb489 shows a similar increase in alanine uptake at 15 nmol/mg LeuT. Additionally, uptake by LeuT-WT with the presence of Nb494 looks similar to the uptake of the liposomes, the control; this indicates the presence of Nb494 inhibits uptake of alanine. Directing the nanobodies against the dopamine transporter, there were similar trends. Specific uptake of dopamine (DA) in the presence of Nb499 was about triple than the DA uptake in the absence of a nanobody. Although less dramatic than Nb499, the presence of Nb489 also showed an increase in the uptake of DA relative to the no treatment group. Finally, the presence of Nb494 had a significantly less DA uptake than the no treatment group, signifying the inhibition of DA uptake. Thus, as clearly illustrated in both uptake reactions, the presence of nanobodies have the ability to affect the activity of the directed transporter.

Since NSS malfunction is directly implicated in psychiatric diseases, several psychoactive drugs specifically interact with these transport proteins (Moncrieff et al., 2013).

There are, however, several side effects to these drugs. Nanobodies having the ability to affect the activity of the transporter without unwanted side effects associated with drug-based therapies, serving as a potential tool to treat psychiatric diseases. Additionally, if monoclonal antibodies can be used for immunotherapy by binding to the dopamine transporter, then nanobodies exhibit potential in the advancement of immunotherapy due to the nanobodies' smaller size, widespread biodistribution, and high stability.

(citations available upon request)

The Effects of Cannabidiol and Lithium on Associative Memory of Drosophila Melanogaster, by Supriya Baskaran, STS Paper Excerpt, Senior

Abstract

The action of several psychoactive medications were tested on the associative memory of fruit flies, as loss of memory is often comorbid with certain psychological disorders or may be associated with treatment.

Cannabidiol (CBD), a component of the Cannabis sativa plant, is used for the treatment of diseases such as PTSD, Alzheimer's, and Schizophrenia. Its effect on memory is still unclear. For this, wild type fruit flies ingested CBD oil or water. The flies were trained to associate an odor with sucrose, and then tested using a T-maze apparatus. A performance index was calculated based on how well the flies learned/remembered to associate an odor with sucrose. This experiment showed that CBD had minor effects on the memory

of D. melanogaster.

For the second part of the study, the action of lithium, used previously to treat bipolar disorder and to improve neurodegenerative diseases, was investigated similarly. One of the possible action mechanisms of the lithium ion may be on the dopamine transporter, a symport using Na+ as a chemiosmotic force. Clomipramine is a tricyclic antidepressant that inhibits the reuptake of dopamine by blocking the dopamine transporter specifically. In this study, fruit flies were given lithium, or lithium with CMI, and then tested on their memory. A significant increase in associative memory was shown when D. melanogaster ingested lithium. This effect was partially suppressed with the combination of Lithium and CMI in a dose dependent manner, suggesting the involvement of the dopamine transporter. More trials will be needed to confirm if this trend is a real effect.

Introduction

Memory is the mechanism through which the mind stores and remembers information. It is a fundamental process, that is necessary for survival. Moreover, memories greatly influence who we are as people, whether it be the decisions we make, our opinions on certain subjects, or just the way we handle different situations.

There are different forms of memory that involve different neurological processes (Dudai, 2002). If memory were to be classified using duration, then it can be split into two categories: short term memories (STM), and long-term memory (LTM). Short term memory refers to the mechanism that holds sensory details, such as smell and noises, and other cognitive information for a small amount of time (Mollet, 2008). These memories typically last for a few seconds in humans. Short term memory is also often times referred to as working memory (WM). While STM is used to describe the maintenance of information that lasts a few seconds, WM is used for the maintenance and

manipulation of the information gathered, for the same brief amount of time (Aben, Stapert, & Blokland, 2012). Previous studies definitely show an overlap between short term memory and working memory, however they may not be identical, like some literature suggest (Aben et al., 2012). Long term memory however is accepted as a completely different entity than STM or WM. Long term memory (LTM) is information retained by the human brain for more than a couple of days up until death.

Associative Memory

Associative memory is memory formed through associative learning. This includes classical and operant conditioning as well as emotional and skeletal responses (Brem, Ran, & Pascual-Leone, 2013). Associative learning/memory falls under procedural and non-declarative memory; which occurs predominantly in the cerebellum, striatum, neocortex and is mostly implicit (Brem et al., 2013). In an operant conditioning procedure the interval between training sessions, as well as the repetition of training sessions determines whether that memory will be stored as short term memory or long term memory (Susan Sangha, Andi Scheibenstock, 2003). If training is very spaced out and does not occur often then the organism trained will develop STM. Whereas, if the training sessions are frequent and occur daily then the associative learning will be input into LTM.

Long Term Memory

The formation and recollection of long-term memory is a complicated process that involves several steps including encoding, consolidation, storage and retrieval. The encoding process takes place in the brain, and occurs when neurons fire rapidly, due to your senses perceiving an important or traumatic event (Tulving, Kapurt, Craik, Moscovitch, & Houlet, 1994). When neurons are fired rapidly, the experience becomes more intense and you are more
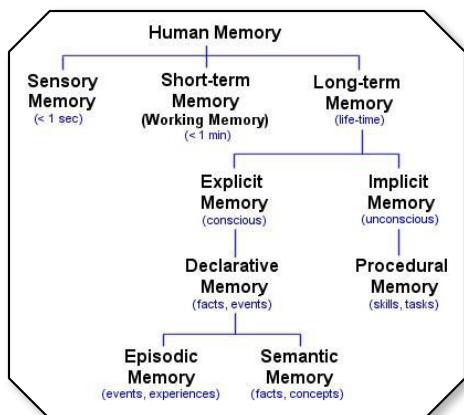
likely to remember that particular event. This biological conceptualization of a memory is referred to as an engram (Ramirez, Tonegawa, & Liu, 2013). Episodic memory is a type of explicit and long-term memory, that allows humans to recall personally experienced events (Tulving et al., 1994). Although there are several reliable sources that have conducted studies and obtained results about variables affecting the encoding and retrieval processes, scientists needed the positron emission tomography (PET) scan to understand the neuroanatomical correlates of the encoding process (Tulving et al., 1994). Tulving and his associated conducted a study utilizing the PET scan to determine that there was correlation between blood flow and memory encoding. During the study they observed that while the brain was engaging in "deeper" encoding activity there was a prominent increase in blood flow to the left prefrontal region, but no significant change on the right hemisphere of the prefrontal region (Tulving et al.,1994). This helped scientists cement the idea that the prefrontal cortical regions are involved in episodic memory, and that there is a prefrontal hemispheric asymmetry in the encoding and retrieval processes of memory.

After an engram for long term memory is created through the encoding process, the next step in long-term memory is the consolidation process, which is vital for long term memory. Consolidation is the process under which a memory trace is stabilized after first being acquired (Ramirez et al., 2013). Studies show that long term memory consolidation is highly dependent on the hippocampal cortex (Moscovitch & Addresses, 1998). The brain contains millions of neurons, and each neuron contains thousands of synapses. All these neurons and synapses are connected together to form a complicated network that can be utilized to store and transport information. New studies have determined that the acquisition and consolidation of memory are dependent

on synaptic plasticity (Dudai, 2002). Synaptic plasticity is the ability of synapses to strengthen or weaken over time, in correlation to the amount of activity they endure.

Neurological Disorders Affecting Memory

Schizophrenia is a neurological disease that has a deteriorating effect on memory. Schizophrenia is a mental disorder characterized by abnormalities in the perception or expression of reality, usually manifesting itself in hallucinations, or disorganized speech. "Noise" in the brains of schizophrenics also results in memory loss, resulting in difficulties in day to day functioning and learning. This debilitating mental illness affects 1% of the total human population, which is approximately 75,270,000 people (Millier et al., 2014). Although the disease is fairly prevalent, the molecular etiology of schizophrenia is largely unclear, making it hard to find treatments that combat both the memory loss aspect as well as the hallucination aspect of the disease.

Cannabidiol

Cannabidiol is a proposed treatment for schizophrenia. Cannabidiol (CBD) is a primary constituent of the Cannabis sativa plant that makes up approximately 40 % of the plant. Δ9-tetrahydrocannabinol (THC) can have antipsychotic effects and has been proven to disrupt working and episodic memory in humans and animal models (Ibeas Bih et al., 2015). Meanwhile the second largest component of C. sativa, CBD, does not impair cognition and has been shown to combat against the memory deficits caused by THC and improve overall memory (Englund et al., 2013). Recently, Cannabidiol is being looked into more as a treatment for neurological disorders such as PTSD, schizophrenia, epilepsy, and Alzheimer's disease because of its anti-inflammatory, and neuroprotective properties.
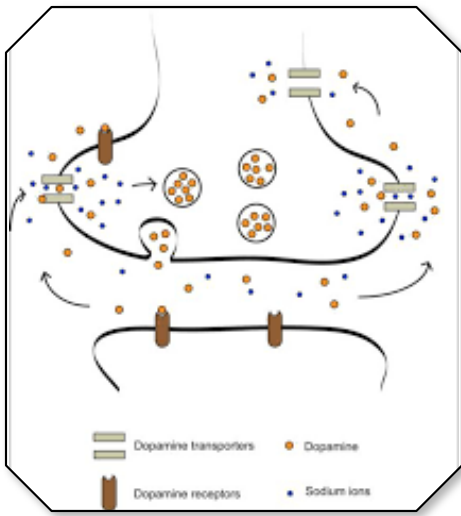
Lithium

Lithium has been used for pharmacological purposes, such as for treating depression, beginning in the

nineteenth century (Shorter, 2009). Since then it has become one of the main treatments for bipolar disorder. Although lithium has been around for several years, lithium's specific mechanism of action in mood regulation is yet to be elucidated (Won & Kim, 2017). It has been previously discovered that lithium directly inhibits glycogen synthase 3β (GSK3β), and has various effects on neurotrophic factors, neurotransmitters, apoptosis, second messenger systems, and biological systems such as the circadian rhythm and hypothalamic–pituitary–adrenal (HPA) axis, all of which underlie lithium's therapeutic benefits (Won & Kim, 2017). The GSK-3 pathway is crucial for supporting synaptic plasticity; as decreased GSK-3 activity corresponds directly to greater levels of cellular resilience and neuroplasticity. Lithium has been found to phosphorylate (GSK3β), inhibiting the action of GSK-3 pathway, which in turn has antimanic and antidepressant effects (Martinowich, Schloesser, & Manji, 2009). Brain-derived neurotrophic factor (BDNF) has been studied extensively for its involvement in neuronal maturation, differentiation, synaptic plasticity, and long-term memory consolidation, and is highly expressed in the cerebral cortex and

hippocampus (Dwivedi, 2009). Previous studies have reported that low BDNF levels correlate to increased bipolar depression and mania (Cunha et al., 2006; Machado-Vieira et al., 2007). Lithium has been suggested to prevent cellular degeneration through BDNF upregulation, with chronic lithium treatment shown to increase BDNF (Won & Kim, 2017).

Lithium may also act upon the dopamine transporter. Previous research suggests that lithium may compete with sodium as the dopamine transporter uses the electrochemical gradient of Na+ as a source of energy for the transport of dopamine. It has been shows that lithium may act on the dopamine transporter, however this relationship has not been explored extensively (Personal Communication Dr. Mathias Quick). Dopamine transporters are part of a larger group of reuptakes (dopamine, serotonin, and norepinephrine) (Campell & Reece, 2005). Leucine is the bacterial homologue of the dopamine transporter, that has been used extensively to characterize this group of transporters. The Na+ is cotransporter with dopamine (DA), thus terminating the action of this transmitter in the synapse. Clomipramine (CMI), a tricyclic antidepressant, has been shown to bind specifically to the dopamine transporter and inhibit the reuptake of DA. It has been extensively used to understand how the transport of dopamine is regulated.

Drosophila

One common organism used to examine the molecular and cellular mechanisms of neurological disorders is the Drosophila Melanogaster, or the fruit fly (Lessing & Bonini, 2009). Drosophila have distinct brain structures known as mushroom bodies (MBs) which are critical for the associative learning and memory of olfactory stimuli; and is the equivalent to the hippocampus in humans. The memory of fruit flies has been researched extensively using conditioning. This is because, almost 75% percent of human disease-causing genes have a functional homolog in the fly and observing learning/memory in fruit flies can help humans better understand human memory and the effect various diseases have on it. Fruit flies are also relatively low in cost and are easy to work with, making them a frequently used model organism (Pandey et al., 2017). Using a variety of genetic techniques several different types of mutant flies can be created and observed (Jeibmann & Paulus, 2009). Additionally, directly studying human brains is against ethics therefore, the use of fruit flies has been vital in neurological research.

Statement of Purpose

In the present study, I analyze how administration of cannabidiol, lithium, and CMI affect short memory using Drosophila melanogaster. I look at how the ingestion of these different treatments affects the ability of fruit flies to learn to associate a specific odor with sucrose and then remember the odor, using a T-maze apparatus. Additionally, by utilizing a combination of CMI and lithium my study hopes to provide further insight into how both

chemicals interact. There are many neurological diseases that negatively impact learning and memory in humans therefore additional information on possible treatments for these diseases is crucial.

Hypotheses

Hypothesis One:

The administration of cannabidiol will improve learning and memory in wild type fruit flies; the CBD will act in a dosage dependent manner

Hypothesis Two:

The administration of lithium will improve learning and memory in wild type fruit flies.

Hypothesis Three:

CMI will act as an inhibitor of lithium and will decrease the impact made by lithium on memory; the CMI will act in a dosage dependent manner

Methods (Excerpt)

Affirmative Olfactory Conditioning (Data Collection)

To test short-term memory, Drosophila melanogaster are taught to associate a certain odor with sucrose (reward) and the other odor with water (no reward). When the fruit flies are tested, if most go toward the odor that was associated with the reward then it can be assumed that associative learning occurred, and the flies are able to remember which odor has the reward. For the training, the flies are first transferred to a training tube containing a sucrose filter paper, that is attached to the MCH odor, and are left there for two mins. The test tubes are then disconnected from the MCH odor and the flies are given air for thirty seconds. Next the flies are transferred to another training tube, this one containing a water filter paper and are
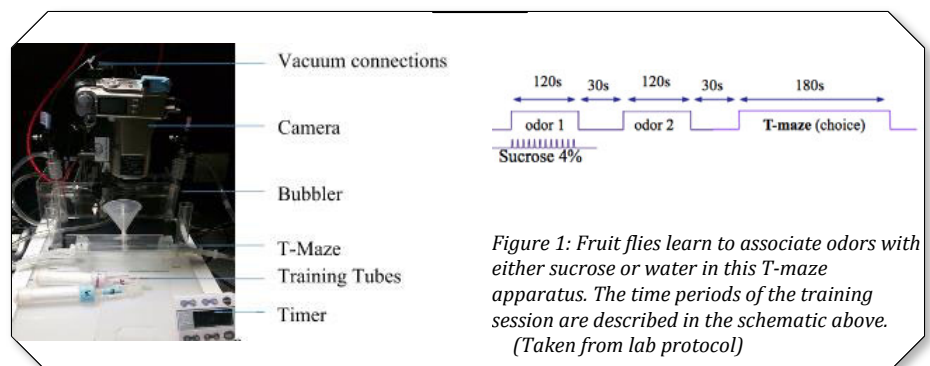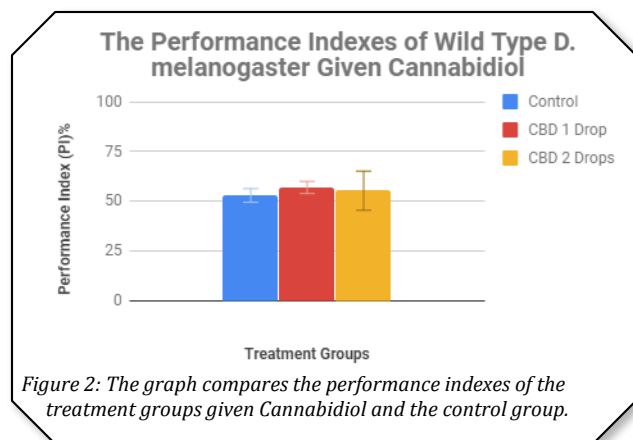

Figure 1: Fruit flies learn to associate odors with either sucrose or water in this T-maze apparatus. The time periods of the training session are described in the schematic above. (Taken from lab protocol)

Figure 2: The graph compares the performance indexes of the treatment groups given Cannabidiol and the control group.

attached to the BA odor for two mins. Afterwards, tubing is disconnected from the BA odor and the flies are allowed to rest for thirty seconds. Then they are transferred into the T-maze apparatus, which is attached to both the BA and MCH odors. The flies are placed inside the chamber and are given three minutes to pick which odor to go toward. After three minutes a picture is taken and the number of flies going toward each odor is recorded. The same process is then repeated with flies given the same treatment, with the only exception being that the flies are trained to associate the opposite odor with sucrose. This ensures that the difference in odors does not affect the associative learning/memory of the Drosophila melanogaster. The training process was repeated five times for each treatment group.

Results

There was no significance monitored between the group of flies given water, the ones given one drop of cannabidiol and the group given two drops of cannabidiol. Fruit flies given CBD tended to do a bit better than the control group, and CBD given only 1 drop performed better than those given 2 drops. However, there was no significant difference found. Meanwhile, fruit flies given lithium had a significant increase in their performance indexes when compared to the flies given just water. Although there was no significant difference found between the group given lithium and the other treatment groups, the trend in the graph suggests lithium performed better than the other groups (Statistical

tables available upon request). Additionally, the trend in the graph shows that the group given a lower dose of CMI in addition to lithium performed better than the one given a higher dosage of CMI, albeit not by much.

Discussion and Conclusion

Although previous literature has indicated that cannabidiol consumption may improve memory, the experiments failed to show that CBD improved associative short-term memory in wild type Drosophila melanogaster. The groups given cannabidiol did however have a slightly higher performance index than the group given just water, and the group given less CBD had a higher average than both the control group and the group given 2 drops of CBD. Additionally, while monitoring the fruit flies, given the different treatments, it could clearly be observed that the groups given a higher dose of cannabidiol were noticeably more active and had a longer lifespan. To measure this a climbing assay and monitoring of the lifespan of the different groups of flies, could be used to see if there was a significant difference between each group and this is something, I am interested in looking at.

Previous research regarding lithium done on Inc mutant D. melanogaster showed that lithium improved the short-term memory of these mutants. Inc mutant fruit flies experience insomnia, and since lack of sleep leads to decreases in memory, Inc mutants obtained very small and negative performance indexes on the T-maze assay. When these mutants received lithium their performance indexes on the learning/associative

memory assay improved dramatically, suggesting that lithium rescued the deficit of STM in the Inc mutant (personal communication Alaina Otto, ISEF project).

The tests performed in this experiment showed that Drosophila melanogaster who ingested lithium performed significantly better on the T-maze apparatus and had significantly larger performance indexes. This suggests that lithium may improve STM and learning in fruit flies. The antidepressant CMI has been shown to interact with the dopamine transporter and inhibits the transport of dopamine (Personal communication from Dr. Mathias Quick). Additionally, it has also been shown that lithium interacts with the dopamine transporter. The dopamine transporter uses an electrochemical gradient of Na+ to transport dopamine back into the presynaptic terminal. It is a symport (aka co-transport) that uses the Na+ gradient to transport dopamine. Li+ can be substituted for Na+, which is interesting because lithium has been used for treatment for psychological disorders such as depression. Although the exact mode of action of lithium has not yet been discovered. A paper on the structure of Leu-T, a bacterial analogue of the dopamine transporter, showed two binding sites for Na+ (Yamashita, Singh, Kawate, Jin, & Gouaux, 2005). It has been shown that Li+ may compete with Na+ at these binding sites (Personal Communication Dr. Mathias Quick). In this study when CMI was combined with lithium the performance

Figure 3: Is a graph comparing the performance indexes of groups given lithium and lithium combined with CMI. It is important to note that the group given lithium had significantly higher performance index than the control group did.
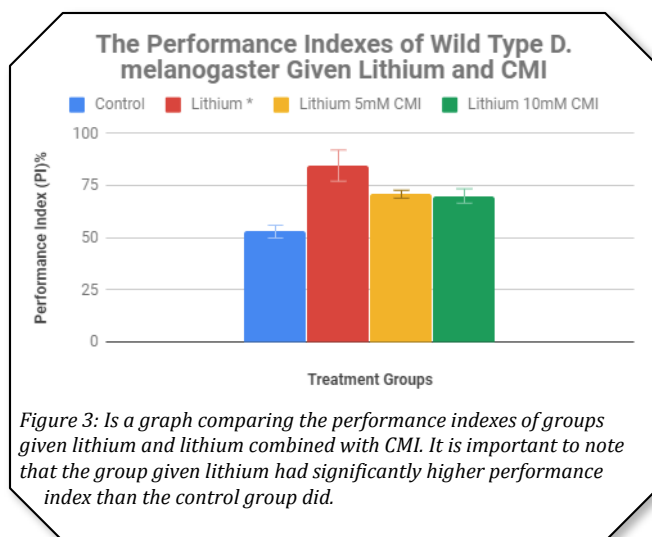
indexes of the flies decreased in a dose dependent manner. With a n=4, I see this trend however it is not significant (p= 0.08). More experiments would be needed with increasing doses of CMI to confirm that this is a real effect. However, it is important to take the limitations and possible errors into account when looking at these results.

The administration of the different treatments may have affected the results. The fruit flies were kept in a vial overnight with the treatment (CBD, Lithium, or CMI) soaked into a Kimtech wipe. This may not be the best way to administer the treatments as it is possible that not all the flies ingested the same amount of treatment. Additionally, NuLeaf CBD oil was used instead of pure cannabidiol, which may impact the results.

Furthermore, these experiments were conducted over a several week span and each time new odors were created. Although the same measurements of each chemical were used it is possible that the odors differed slightly which could have affected the behavior of the fruit flies.

Moreover, during the training process some flies escape the T-maze apparatus and some die, and although this is taken into account when determining the performance index this is also another limitation of the study as some groups have more flies than others. The study is limited in that there is not a way to determine whether this assay is truly reflective of how the different treatments affect overall memory.

Future Studies

Due to insufficient data it is not possible to conclude if the administration of CMI, lithium, or cannabidiol affected the morphology of the fruit fly brain, specifically the dopamine system found in flies. Therefore, for future studies fruit flies given the different treatments should be dissected and stained to compare the dopaminergic neurons. The study also found a general trend of higher doses of CMI adversely affecting short-term memory, however it failed to show significant difference. The concentrations of CMI used were also based on studies done on mice and it is possible that a different concentration would work better for D. melanogaster. The study is ongoing and it is possible that additional data could confirm the trend found that higher doses of CMI counteract the effect of lithium on STM. I would like to look at how CMI alone, and a higher dosage of CMI with lithium affects the associative memory of fruit flies.

The administration of the various treatments could also be changed to test if long term administration of these treatments affected learning and associative memory in fruit flies. This could be completed by creating food vials with the treatments and testing the flies after multiple days. This would ensure that all the flies would receive the treatment, as it is mixed in with their food. These food vials should also be monitored, and the amount of living flies should be counted each day, as this can provide further insight into if the treatments affect lifespan. Tests should also be done to see the effect of lithium, CMI, and cannabidiol on LTM of D. melanogaster.

In the future, this study should also be completed on different Drosophila mutants such as DISC1, Psn, and INC. The treatments used in this study may affect the fruit fly mutants differently and can offer insight on if cannabidiol, lithium, and Clomipramine could be used as treatments for various neurological diseases.

Why This is Important

This study provides evidence to suggest that cannabidiol, lithium and CMI improve learning and memory to various degrees. Many neurological diseases such as schizophrenia, Alzheimer's, PTSD, and depression affect learning and memory in humans. This study provides additional information about how these treatments may affect memory. This could be utilized as a good basis to start additional research on whether these chemicals are suitable treatments for various diseases.
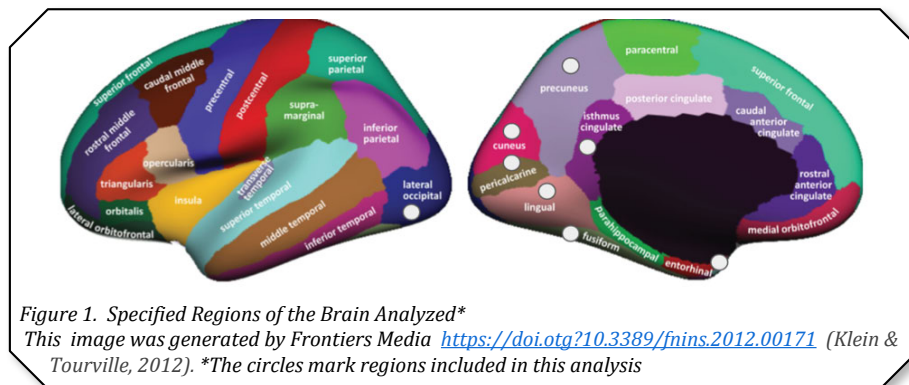
(citations available upon request)

Examining Morphological Associations of the Visual System Related to the Reading Ability of Young Children, by Hailey Kissner, STS Paper Excerpt, Senior

Abstract

This study investigated brain structure as it relates to reading and the dual deficit model of dyslexia. In this research, differences associated with brain structure and reading scores were found, which could relate to dyslexia. Regions in the visual system were specifically focused on and scores were analyzed on both the Rapid Automatic Naming (RAN) tests and Letter Word (LW) identification tests which require visual processing. Dyslexia is hypothesized to have three subtypes including (RAN), phonological awareness deficits, and double-deficit consisting of both RAN and

Figure 1. Specified Regions of the Brain Analyzed*
This image was generated by Frontiers Media https://doi.otg?10.3389/fnins.2012.00171 (Klein & Tourville, 2012). *The circles mark regions included in this analysis

phonological awareness. RAN, the ability to rapidly identify items on a page has been shown to parallel cognitive and neural demands of reading because the brain must automatically recognize items on the page. Lower RAN and phonological awareness scores have been shown as a strong indicator of poor reading. Based on current research, MRI results have shown atypical surface area, cortical thickness, and volume in multiple brain regions which have been associated with lower reading scores. Participants in this study were 5-9 years old and underwent an extensive set of standardized reading, language, and cognitive measures. An MRI scan was obtained for each subject and analysis was performed on regions of the brain involving the visual system. Associations were found among cortical surface area in several regions of the brain and scores on LW and RAN tests.

Introduction

One of the most prevalent learning disabilities is dyslexia, affecting 4–10% of the population (Pijpker, 2013). According to the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), "Dyslexia is an alternative term used to refer to a pattern of learning difficulties characterized by problems with accurate or fluent word recognition, poor decoding, and poor spelling abilities." (p.67, DSM-5). Although dyslexics struggle with reading and writing, the disability does not impact the individual's overall intelligence. In fact, many dyslexics are successful despite adversity. Visual processing is one important component of reading, engaged in both reading and RAN, which is linked to reading ability. The following study examines brain structure and performance on these assessments. Since reading relies on the visual system to process text, the brain regions related to this aspect of reading is of interest.

Literature Review

One leading hypothesis is that children with dyslexia is comprised of three subtypes: RAN deficit, phonological awareness deficit, and double deficit (DD) consisting of both RAN and phonological awareness. To investigate the DD hypothesis, scientists have conducted extensive research. Specifically, researchers have evaluated adolescents in areas related to the specified subtypes through color naming, digit matching, and math expression problems and found that dyslexics performed worse than children without dyslexia. Additionally, dyslexic children scored lower in the reading and word decoding tasks, completed fewer math problems during the math fluency and calculation tasks and had difficulty with symbolic number comparisons (Träff, Desoete, & Passolunghi, 2016). Norton et al. (2014) assessed a group of poor readers and normal readers while completing tests of phonological awareness and RAN during brain imaging with functional MRI (fMRI) to assess the DD hypothesis. Individuals with dyslexia showed a dissociation between left inferior frontal and inferior parietal regions and right cerebellar lobule VI, which are brain regions sensitive to RAN and phonological awareness, respectively (Norton et al., 2014). The authors found that the DD group scored the lowest on the reading ability tasks. There was little difference between the reading ability according to the testing of the DD and single phonological deficit individuals (Norton et al., 2014).

This study also demonstrated that the RAN deficit group performed better than both the phonological deficit and the DD groups on reading assessments. RAN is one reliable indicator of literacy and is characterized as the capacity to quickly list an assortment of repeating items presented in a visual array including numbers, objects, letters, and/or colors. Amid a RAN test, the time taken to name the visual stimuli shown is recorded and is thought to parallel the cognitive and neural demands for

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Yale Child Study Center
and Haskins Laboratory
Yale University**

Table 1. *RAN and Brain Region Correlations†*

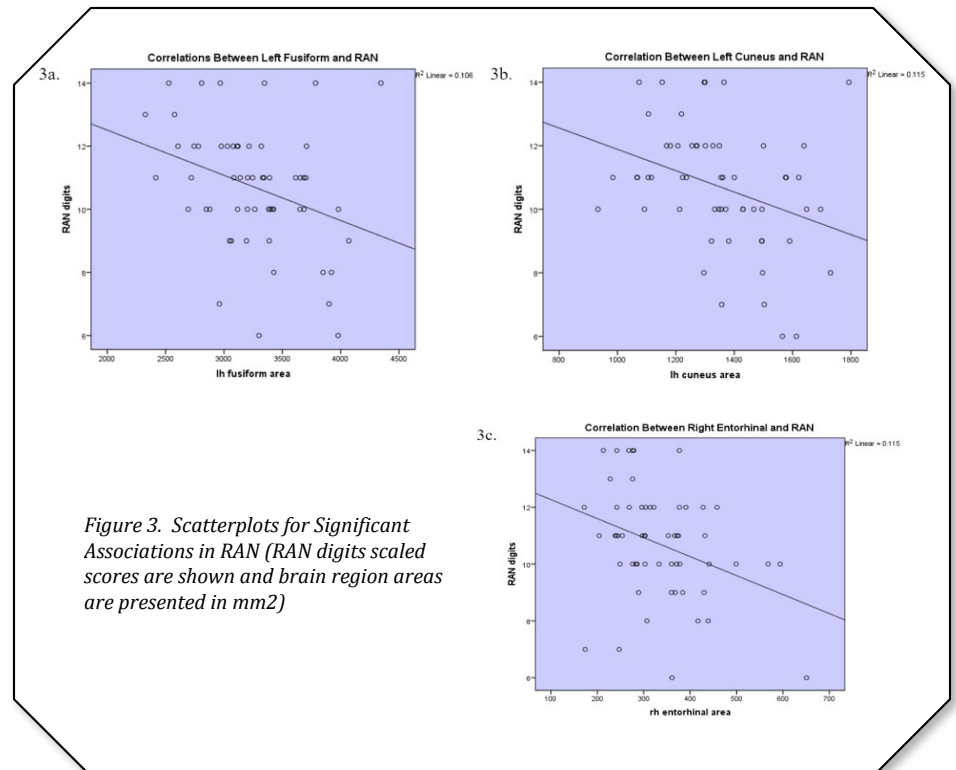| Brain Regions	Correlation Coefficient *r*	Significance *p* value	DF
R entorhinal area	-0.334	0.012*	54
R fusiform area	-0.239	0.076	54
L fusiform area	-0.324	0.015*	54
L cuneus area	-0.335	0.012*	54

*p value is statistically significant
† n=57

proper reading because the brain automatically identifies the words on the page just like RAN in the brain quickly responds to visual stimuli. In reading, RAN serves as a strong indicator of reading achievement (Ozernov-Palchik & Gaab, 2016). RAN is known to be associated with brain regions including the left inferior frontal gyrus, left posterior middle frontal gyrus, bilateral inferior occipital areas, and was less strongly correlated with the left parietal and right frontal areas (Pugh et al., 2001; Norton et al., 2014). Another strong predictor of reading ability is phonological awareness, a skill used to recognize and manipulate parts of a spoken language such as words and syllables. This skill has been deemed an indicator of dyslexia when compromised. During a phonological awareness test, individuals must complete tasks related to the sounds of words like rhyming and sound-matching. Several studies have looked at phonological awareness and its associated brain regions like the superior temporal gyrus, middle temporal gyrus, superior frontal gyri, and fusiform gyrus (Morken, Helland, Hugdahl, & Specht, 2014; Kovelman et al., 2012). Similarly, the left inferior frontal gyrus and the left middle frontal gyrus have been found to be associated with RAN and phonological awareness (Kovelman et al., 2012). Other brain regions will be important for various aspects of reading since reading is a complex task that requires the support from many cognitive processes.

The brain has two systems associated with reading, the ventral circuit, and the dorsal circuit. The ventral circuit includes occipitotemporal regions (point of contact between the ventral visual stream and middle-inferior temporal) and is associated with speech, fluency, word recognition, phonological reading, silent reading, and naming. For example, the fusiform gyrus or visual word form area is one such structure in the ventral circuit; this region stretches across the basal surface of the temporal and occipital lobes, and has been found to play an important role in word processing (McCandliss, Dehaene, & Cohen, 2003). Also, the lingual gyrus, located in the occipital lobe is a part of the ventral system and plays a role in color perception, and is linked to processing vision, especially its relationship to letters. It is thought to be involved in the analysis of logical conditions (i.e. logical order of events) and encoding visual memories (Bogousslavsky, Miklossy, Deruaz, Assal, & Regli, 1987; Raschle, Chang, & Gaab, 2011). Similar to the other

aforementioned parts of the brain, the lateral occipital cortex (located in the occipital lobe) also in the ventral system has more general sensory functions, but it still imposes important functions such as the processing of tactile and visual information like object processing (Margalit, Shah, Tjan, Biederman, & Keller, 2016). In contrast, the dorsal circuit is located in the temporoparietal areas of the brain and consists of the supramarginal gyrus and the angular gyrus, which are thought to be associated with phonological processing (Pugh et al., 2001). Specifically, the precuneus which is located in the dorsal circuit and part of the front of the occipital lobe was found to be associated with visual-spatial processing (Trimble & Cavanna, 2006). The isthmus, the posterior portion of the cingulate gyrus is also a part of the dorsal system and this region is located at the junction of the forebrain in the parietal lobe which has been associated with emotion processing, learning, and memory (Webb, 2017; Johns 2014; Desikan et al., 2006).

Other areas examined in this study that are not specific to the dorsal or ventral systems, but have more basic sensory functions are the thalamus, cuneus, pericalcarine gyrus, and the entorhinal

Figure 3. Scatterplots for Significant Associations in RAN (RAN digits scaled scores are shown and brain region areas are presented in mm2)

cortex. The thalamus, the dorsal part of the diencephalon which is mainly interconnected with the cerebral neocortex, is located between the cerebral cortex and the midbrain (Betts et al., n.d). This region is responsible for relaying motor and sensory information (including visual signals) to the cerebral cortex. The cuneus, located between the calcarine fissure and the medial part of the parieto-occipital fissure in the occipital lobe, is responsible for visual processing such as spatial frequency, orientation, and motion (Gray, 1918). Another general sensory region is the entorhinal cortex, located in the temporal lobe, which serves as the interface between the hippocampus and the neocortex (Joseph, 2000). This area is important for processing impulses from the eye and ear and plays a role in memory formation navigation. Lastly, the pericalcarine cortex or primary visual cortex of the occipital lobe is responsible for receiving and processing impulses from optic nerves (Visual Processing: Cortical Pathways, n.d). The aims of this study are to investigate brain regions that may support visual aspects of reading because deficits in visual processing contribute to poor reading (Margalit et al., 2016). Therefore a selection of the specified areas of the brain described above will be analyzed.

The hypothesis that cortical thickness and surface areas in brain regions important for visual aspects of reading will be lower in children with lower reading/RAN scores. Specifically, the hypothesis is that MRI results and associated behavioral data with lower reading scores will have less surface area and cortical thickness in the precuneus (Trimble & Cavanna, 2006). In contrast, participants who performed better on the reading tasks most probably will have more surface area and cortical thickness in those specific regions. According to other studies comparing normal readers' scores on visual learning tasks to dyslexics, there was a major deviation between the performance of dyslexic individuals and non-dyslexics; therefore,

Table 2. *LW and Brain Region Correlations†*

Brain Regions	Correlation Coefficient r	Significance p value	DF
R fusiform area	-.340	.010*	54
R precuneus area	-.250	.063	54
R cuneus area	-.227	.092	54
R lateral occipital area	-.246	.068	54
L cuneus area	-.259	.054	54
L fusiform area	-.418	.001*	54
L lateral occipital area	-.303	.023*	54

*p value is statistically significant
† n=57

suggesting dyslexics have a deficit with visual-spatial processing (Richlan, Kronbichler, & Wimmer, 2009). This deficit in dyslexia may be linked to the reduced gray matter structure of the precuneus. The fusiform gyrus or visual word form area showed more activation in normal readers while reading compared to dyslexics who had a minimal indication of activation in this area of the brain (McCandliss et. al., 2003). A lack of activation in this region of a dyslexic's brain can be related to reduced surface area and cortical thickness and may look similar to an individual in this experiment with lower scores on reading tests. In one study, lesions located in the temporal lobe (area of entorhinal cortex) resulted in impaired recognition memory for tactile and recurring visual stimuli. Since dyslexics have these impairments, the cortical thickness and surface area of the entorhinal cortex may be smaller in individuals with lower reading scores, which explain these symptoms (Schröder, Haak, Jimenez, Beckmann, & Doeller, 2015). The lateral occipital cortex is associated with general visual processing. Research has demonstrated that dyslexia has both an auditory and visual perception deficit (Margalit et al., 2016); therefore the hypothesis that this deficit may be linked to the reduced gray matter structure (surface area and cortical thickness) in the lateral occipital cortex of the poorer readers. Studies have shown differences in brain

structure in the lingual gyrus, thus, it is proposed that both the surface area and cortical thickness are lower in participants with lower reading scores (Raschle et al., 2011). Relating to the cuneus, a study comparing a dyslexic brain to typical readers, activation occurred for the average readers in the cuneus while reading, while there was no task-related activation in the brain of the dyslexic readers. This lack of activation may be related to reduced surface area and cortical thickness and may correlate with lower reading scores (Olulade, Flowers, Napoliello, & Eden 2015). In the pericalcarine cortex or primary visual cortex individuals with dyslexia showed reduced activation compared to normal readers (Demb, Boynton, & Heeger, 1997). Specifically, this lack of activation may account for a lacking amount of surface area and cortical thickness in the pericalcarine cortex and may be associated with the lower reading scores. According to a study by Paul et al, the isthmus of the dyslexic brain is smaller than in typical readers (Paul, 2011). Therefore, based on this it is proposed that surface area and cortical thickness will be lower with the lower reading scores thalamus, the dorsal part of the diencephalon which is mainly interconnected with the cerebral neocortex between the cerebral cortex and is responsible for relaying motor and sensory information to the cerebral cortex. Hence, the amount of volume in

this region may be lower with lower reading scores because it has been shown in neuroimaging dyslexia studies that thalamic anomalies are present (Fan, Davis, Anderson, & Cutting, 2014).

Goals of Study

The following study seeks to identify associations among brain structure of various regions in the visual system and reading ability. Additionally, by performing split gender analysis, morphological differences across genders may be identified. Such research will determine if there are structural associations with lower reading scores which may correlate with dyslexia. These results may provide a neural marker for children with reading difficulties and aid in the diagnostic process of dyslexia and related learning disabilities. Additionally, this may assist in identifying aspects of brain structure associated with individual differences in reading.

(...)

Results

There was a significant negative association between the right entorhinal cortical surface area and RAN (R (54)= -.334, p= .012). In the left fusiform, there was also a significant negative association present for the cortical surface area and RAN (R(54)= -.324, p=.015). Additionally, a significant negative association was found for the left cuneus cortical surface area and RAN (R (54)= -.335, p= .012). Finally, there was a trending association between the right fusiform surface area and RAN (R(54)= -.239, p= .076) (Table 1).

There was a significant negative association found for the right fusiform cortical surface area and LW (R(54) = -.340, p= .010). Additionally, a significant negative association was identified for the left fusiform area and LW (R(54) = -.418, p= .001). For the left hemisphere, significant negative associations were found for the lateral occipital area and LW, (R(54) = -.303, p= .023). In the right precuneus, a

negative trending association was found for the area and LW (R(54) = -.250, p= .063). In addition, a negative trending association was found for the right lateral occipital area and LW (R (54)= -.246, p=.068). Finally, negative trending associations were found between cuneus area and LW in both hemispheres: right cuneus (R(54) = -.227, p= .092) and left cuneus (R(54) = -.259, p= .054) (Table 2)

Split Gender Analysis: Cortical Surface Area and RAN

There was a significant positive association between the left isthmus cortical surface area and RAN (R(28) = .476, p= .008) in females. Also, in females negative trending association was found for the left cuneus area and RAN (R(28) = -.335, p= .070). In males, trending associations were found for cortical surface area and RAN in the right hemisphere lateral occipital (R(23) = -.344, p= .092), right entorhinal (R(23) = -.372, p= .067), and left fusiform (R(23) = -.371, p= .068).

Split Gender Analysis: Cortical Surface Area and LW

In females there was a significant negative association between the left fusiform surface area and LW a (R(28) = -.551 p= .002).

Split Gender Analysis: Cortical Thickness and LW

A significant negative association was found between right hemisphere lingual cortical thickness and LW scores n females, (R(28) = -.393, p= .032). Additionally, a significant negative association for the left hemisphere thickness of the precuneus and LW (R(28) = -.337, p= .068) was observed in females. For the left lingual cortical thickness and LW, a positive trending association was found (R(23) = .334 p= .092) in males.

Discussion of Results and Conclusions

A false discovery test for multiple comparisons (Benjamini-Hochsted) to further prove our findings were used. However, the value threshold between 0.0008 and 0.0047, therefore, results

not corrected for multiple comparisons but are significant at the standard threshold of .05. Negative trends were found between brain structure and RAN/LW in various regions. This may be associated with the typical developmental process of synaptic pruning in which stronger connections are enhanced while weaker ones are eliminated, which may reduce the amount of grey matter. Additionally, this may be related to the process of myelination which occurs during development. During this process, axons are like electrical wires so they require insulation just like how your phone charger is coated in plastic. Thus, in the brain, axon wires are coated with myelin, a fatty tissue, in order to insulate the axon and allow for the electrical charge to travel faster and more efficiently to the axon located on the neuron it is trying to communicate with. However, over the course of development, the myelin builds up as one ages more myelinated cells are present in the brain, which builds up in the white matter. In summary, more cells become myelinated so measures of grey matter are reduced. Overall negative associations found between RAN/LW and brain measures may be explained by the typical developmental processes of pruning and myelination reduction or slowing in children with poorer reading scores. This could lead to less efficient processing in brain areas that support reading.

These findings show a negative association between the right entorhinal cortical surface area and RAN. These results indicate that lower RAN scores were associated with a greater surface area in the right entorhinal. This may be explained by processes of synaptic pruning and myelination. Additionally, abnormalities in the right entorhinal are consistent with current research, specifically overactivation was found in dyslexics' right entorhinal (Panogotta et al., 2015).

Another significant negative association was found for the left fusiform area in correlation with RAN

and LW scores. These findings suggest that lower RAN/LW scores are associated with a greater area in the left fusiform and may relate to the idea of pruning and myelination. Current research has found that dyslexics have greater thickness in this region which could support these findings (Ma et al., 2015). Additionally, less cortical gray matter volume was found in the left fusiform in dyslexia (Kronbichler et al., 2008). This is interesting because brain regions in this study found an inverse relationship among the brain measurement (area, thickness or volume) and performance on reading tasks, so this result demonstrates that poorer readers have less volume. However, there are mixed findings in the literature which suggests either a greater or lesser gray matter is linked to better or poorer reading ability. This may be due to differences in methods, participants, age groups, and other confounding factors. The trend present in the study that discusses the thickness of the left fusiform is consistent with these findings that dyslexics which are usually associated with poorer reading frequently have increased brain measures in their visual systems (Ma et al., 2015). Although these data are for cortical thickness and volume, it may still be relevant since it relates to the same region. Also, under activation was found in the left fusiform region in dyslexics and this may be consistent with a deficit in the parallel processing of legal letter strings and expresses an impairment link between visual or other sensory information to higher-order representations (Richlan et al., 2009).

For the left cuneus, a significant negative association was found for cuneus area and RAN which demonstrates that lower RAN scores are associated with a greater area. This may be related to the idea of pruning and myelination. Current research dyslexic children also displayed white matter reduction in bilateral parieto-occipital regions (cuneus and precuneus) irrespective of age (Xia, Hoeft, Zhang, & Shu, 2016).

A negative trending association was found for the right fusiform area and RAN. In future work to replicate the present findings, this region could prove to show a greater area with lower scores and would be consistent with previous research highlighting that abnormalities are present in the fusiform in dyslexia.

There was a significant negative association found for the right fusiform cortical surface area and LW scores. These results indicate that lower scores are associated with a greater area in the region of the right fusiform related to the process of pruning and myelination. According to Kronbichler et al, less cortical gray matter volume was found in the right fusiform of dyslexic individuals. Even though the current findings relate to the area of the right fusiform, abnormalities relating to volume may be relevant since the same region of the brain was evaluated. Research shows that gray matter volume is closely related to surface area (Frye, 2010). Such morphological differences in the dyslexic brain may be due to the processes of pruning and myelination.

In the right precuneus, a significant negative association was found for area and LW scores. This demonstrates that poorer readers had a greater surface area in this region which could be explained by pruning and myelination. An aforementioned paper supports such findings by suggesting that dyslexic children have shown a reduction in white matter in various parieto-occipital regions including the precuneus (Xia et al., 2016). Such findings are relevant to this study since it is consistent with the interpretation about myelination resulting in changes to gray matter. Also, previous research suggests that increased activation in the precuneus was associated with children with spelling and reading impairments compared to the normal performing children; this further supports the notion that abnormalities are present among the dyslexic brain (Gebauer et al., 2012).

A significant negative association was found for both the right and left lateral occipital area and LW scores, which demonstrates that lower scores are associated with a greater area. Similarly, this may be due to pruning and myelination. It has been documented that there are abnormalities in the anterior aspect of a dyslexic's lateral occipital gyrus which further implicates this region as having certain structural differences for individuals with dyslexia (Shaywitz et al.,1998).

Trending associations were found for the left and right cuneus. In the future these results may prove to be significant.

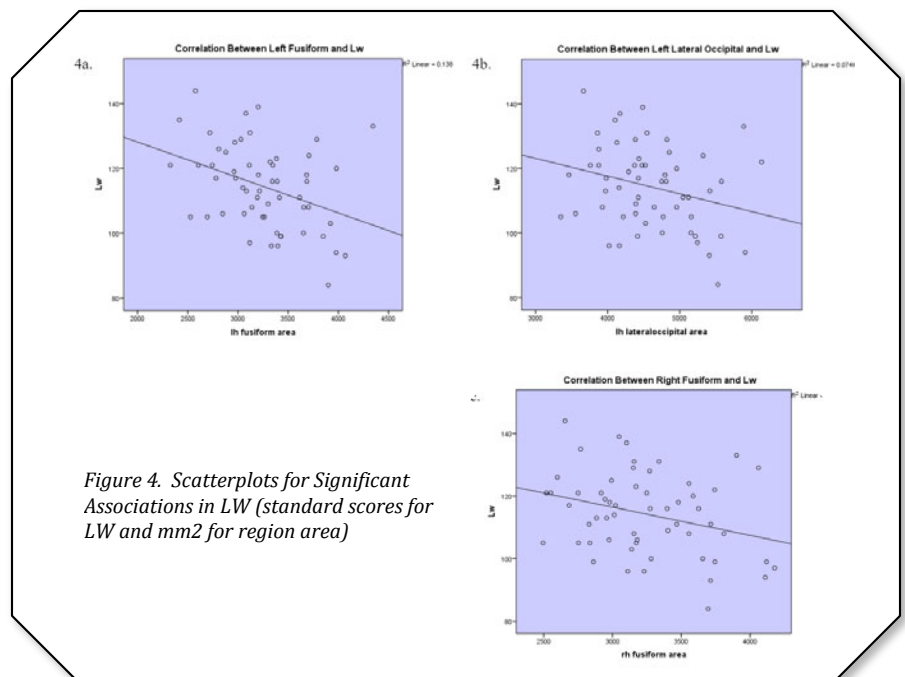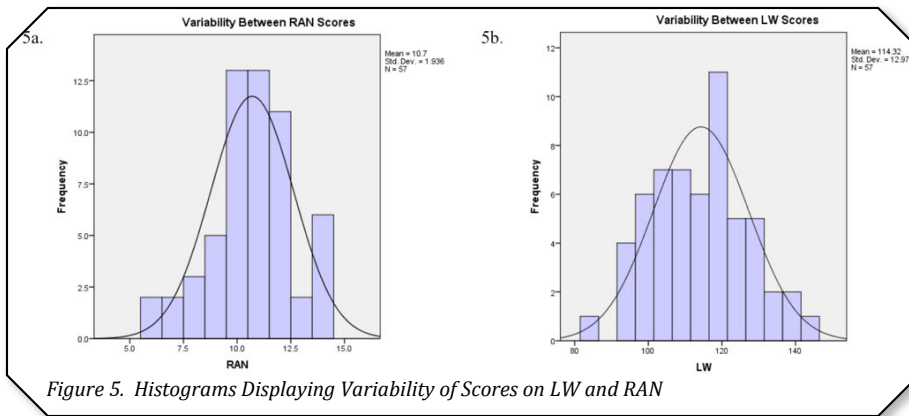In the split gender analysis, there were more specific differences found for

Figure 4. Scatterplots for Significant Associations in LW (standard scores for LW and mm2 for region area)

Figure 5. Histograms Displaying Variability of Scores on LW and RAN

females. Meanwhile in males, the data was consistent with the overall findings. Results from males may be driving associations in most of the published literature. Therefore, future research should focus on including gender as a variable because there could be differences in male and female brains as they relate to reading. With this information, the morphological differences found across genders may be relevant to diagnoses associated with these differences.

Limitations

The mental status of the children during testing was not reported; therefore, the test results may not be an accurate representation of their performance. Another notable consideration was the high averages on the RAN and LW tests. Specifically, in this study the mean score for LW and RAN was 114.32 and 10.7 while the normal mean is 98.8 and 10, respectively (Figure 5;

McGrew, Laforte, & Schrank, 2014; Wagner, 1999). These scores on the LW test show that the sample lacks variability. Meanwhile, there is not a drastic difference for the mean of the RAN scores in this study versus the standard normal, but this is still important to note. In the future, it would be interesting access the relationship between volume and area along with the relationship between thickness and area. In addition, further research would relate to the implications of activation in fMRI with brain size may be helpful. In the future, understanding the participants diagnostic status associated with dyslexia or learning disabilities and apply the current findings in correlating those results with brain scans of those diagnosed with dyslexia may prove to be helpful in aiding the development of a neural marker for children in diagnosing dyslexia and related issues.
(citations available upon request)

Implicit Racial Bias Affecting the Health Care System, Kimberly McKoy, STS Paper Excerpt, Senior
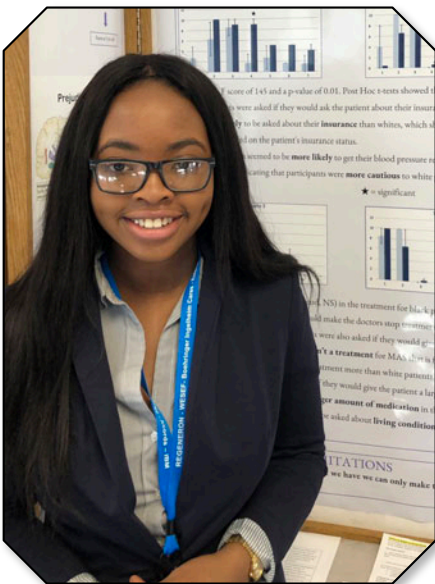
Abstract

Implicit bias is the unconscious bias that someone might have against another group. Everyone has an implicit bias and our biases are derived from societal stereotypes and the environment in which we grow up. The African American infant mortality rate is six times higher than that of a White American however, socioeconomic factors, occupation and living conditions are not the reason for this mortality rate, but, unfortunately, this mortality is caused by racial discrimination. Doctors who have implicit racial bias may unconsciously affect the way they form medical decisions. Doctors will make treatment decisions based on race instead of the patient's description of pain. I constructed a clinical survey that was distributed to medical students and physicians that specializes in the area of Obstetrics and Gynecology and Pediatrics. The surveys included case studies that presented certain scenarios but had two different photos of races, to see if there was a discrepancy in treatment decisions based on race. Trends show that there may be implicit racial bias in the treatment decisions, but however, due to the small number of participants, significance could not be established. Trends show that African Americans are may be given less pain medication, and riskier procedures compared to White Americans. The insurance status of the African American patient might also interfere with the treatment that the individual may receive.

Introduction and Literature Review

Bias is the negative or positive evaluation of someone from group that differs from the evaluator. Differences in biases are indirect and direct (Blair, I. V., et. al, 2011). An example of an indirect bias is called implicit bias, the unconscious negative or positive evaluations of someone from another group. An example of direct bias is called prejudice bias, and sometimes known as explicit bias, which is the conscious negative evaluation of someone else. Explicit and implicit bias differs from the underlying thought process from the evaluator (Blair, I. V., et. al, 2011). Explicit requires the exact judgment of someone followed by a reaction, which could result in an inappropriate comment or gesture.

Implicit bias influences a person's perception, memory, and behavior, thus it can operate in an intentional manner and can be activated by situational cues, for instance, skin color or accents. (Blair, I. V., et. al, 2011).

Implicit racial bias is the unconscious racial bias of someone towards another group. This develops through cultural differences based on stereotypes that our society portrays. Due to Implicit racial bias acquiring in our brains, everyone has it and it affects everything that we do. It is likely to be originated in the amygdala, part of the limbic system, that is activated when a person is formulating an implicit bias (Kubota, J. T., et. al., 2012). The amygdala comprises a group of nuclei for cognitive functions and classical fear conditioning (Kubota, J. T. et. al., 2012). As we evolved from tribes, we have the ability to distinguish from other tribes or racial groups as a defense mechanism (Johnston, 2017). Explicit racial bias is an intentional bias that someone has of another group and is derived from personal opinions based on cultural stereotypes. Prejudice is the consequence of racial stereotypes that are applied in our thought process (Devine, P. G., 1989). Implicit racial bias contributes to differences in medical treatment due to racial differences (Biernat M, and Manis, M 1994). This encompasses all races but most studies have focused on possible biases between White Americans and African Americans.

The implicit racial bias of doctors seems to affect the lives of African American infants. More than 2,300 African American infants die each year in the U.S before their first birthday (Carpenter, 2017). It's found that African Americans are more than six times more likely to lose their infants compared to White Americans (Carpenter, 2017). Despite, income, socioeconomic status, and occupation, the factor of racial discrimination through implicit racial bias increase the mortality rate of African Americans, such that doctors who have implicit bias may make treatment decisions based on race instead of the patient's description of pain (Greenwald AG, et. al., 2012). There are many racial differences in medical diagnosis (Ansell and McDonald E., 2015). Additionally, cognitive stress can lead a caregiver to have a large implicit bias (Devine et al., 2012), and this can occur especially if there are racial differences between a caregiver and their patient (Schaa et al., 2015). Vulnerable patients, elderly, mentally ill, overweight, women, transgender, minority and disabled, who are receiving care, are typically in groups that may be put at risk by the implicit biases of doctors. (FitzGerald, C., & Hurst S., 2017). Patients who get worse health care due to their race and socioeconomic status, are found to have a lower quality relationship with their doctors. This loss of communication often comes from racial bias and differences in cultures between the doctor and the patient (Meltzer LJ, et. al. 2009). Doctors are more likely to not listen to their patients because of a poor relationship and this results in worse care and outcomes.

An African American mother by the name of Simone Landrum was suffering from preeclampsia early in her pregnancy. She experienced many sleepless nights of headaches and back pains, she was alarmed by this feeling and asked her doctor if they can take some tests. But her doctor recommended her to just take Tylenol and never took the time to give her a test to see if it could be a serious condition. This implicit racial bias led to the death of her baby girl and almost to her death (Villarosa, 2018). Implicit racial derives from lack of day to day interracial and intercultural interactions (Ansell D, and McDonald E., 2015). Physicians with implicit bias tend to have different treatment outcomes based on the racial differences of patients (Gaertner et al., 1994). For example, black patients are assigned less pain medication compared to white patients but are diagnosed with more diseases (Greenwald AG et. al., 2012). These disparities are caused by differences in race and insurance status (Ansell D, and McDonald E., 2015). Minorities are more likely to receive less recommended treatments for diseases from ranging from AIDS to cancer. (Ansell D, and McDonald E., 2015). There is a direct relationship for a physician's implicit bias linking to the mistrust of black patients. For instance, an Arizona mother died from the amount of anesthesia that was given prior to fetal surgery from the implicit bias from the anesthesiologist, who thought she smoked marijuana because she wore dreads (Martin, 2017). Many treatment decisions that are made by physicians are based on race instead of the patient's description of pain, which increases the mortality rate of African American infants (Greenwald AG et.al., 2012). These studies show the relevance of racial bias today and its existence needs to be understood so that it can be prevented. Previous studies have discussed the statistics of the infant mortality rate of African Americans. Socioeconomic status, employment, and other factors are not a cause of the rate of discrimination, as they were controlled for in these studies. However, a link between the mortality rate and the racial discrimination that patients are experiencing has been demonstrated in these studies. If physicians have a large implicit bias, then there will be a disparity in the

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Questions	Scenario 1: Anemia	Scenario 2: Preeclampsia	Scenario 3: MAS	Scenario 4: VBAC
Q1	Advise bed rest	Ask about stress	Give pain medication	Give pain medication
Q2	Clinical question	Recheck blood pressure	Clinical question	Clinical question
Q3	Clinical question	Clinical question	Clinical question	Advise bed rest
Q4	Ask patient about her insurance	Advise bed rest	Clinical question	Clinical question
Q5	Ask patient about relationship with father of the child	Clinical question	Clinical question	Ask patient about her insurance
Q6	Ask about living conditions at home	Ask patient about her insurance	Ask patient about her insurance	Ask about living conditions at home
Q7	Give patient advice about how to take care of baby	Ask about living conditions at home	Ask about living conditions at home	Ask patient about relationship with father of the child
Q8	N/A	Ask patient about relationship with father of the child	Ask patient about relationship with father of the child	Give patient advice about how to take care of baby
Q9	N/A	Give patient advice about how to take care of baby	Give patient advice about how to take care of baby	N/A

healthcare system, especially increasing the infant mortality rate of black infants because caregivers are not aware of their own bias. Using this hypothesis, the goal of my study is to lower the percentage of implicit bias, in consequence decreasing the mortality rate in the healthcare system, by making physicians aware of their biases. Additionally, this could lead to introducing training in medical schools that will help medical students become aware of their own biases and work on lowering them. If physicians and medical students will become aware of their bias and will be trained to recognize it and prevent it, they will be changing their method of treating patients. (...)

Results

The surveys of Obstetrics and Gynecology and Pediatrics were distributed over social media for participants to complete. Two Versions of each survey were placed on social media, one that had more pictures of black patients, and the other of white patients. They were four surveys in total as for each specialty there were two versions that differed in pictures. For the surveys, we had a total of 9 participants for all four of the surveys. Unfortunately, there were not enough participants for the surveys of Pediatricians, leading us to be unable to

compute a bias score and to use statistical analysis to compare the groups. However, we were able to analyze the survey of Obstetrics and Gynecology and compute scores. Except for scenario 1, there were not enough participants to establish significance and analyze the data with ANOVAs and post-hoc t-tests for the remaining three clinical vignettes, thus our conclusions may only reflect some trends in the responses.

Scenario 1

For each Case study, there was a scenario with a supply of questions on how the participants would agree and disagree with a Likert scale from 0 -10. The first case study described a scenario of a mother who comes in for an ultrasound appointment and complains of being tired and dizzy. The patient has a pale skin color and is short of breath. During her ultrasound appointment, she has a rapid heartbeat and has trouble concentrating. The patient is assumed to have Anemia, but this information is not given to the participants, for

their medical knowledge and bias is tested for the best treatment options. Information is given to the participants who also gets the following seven questions; two that are clinical questions and five that are biased questions. The first question asks if the participants would advise the bed rest. For participants that received version 1 with a white patient answered with a mean of 2.67. For participants that received a black patient answered with the mean of 0. By looking at the data, whites have a higher mean than blacks, concluding that white patients are more likely to be put on bed rest. Participants were asked if they recommended that the patient needs blood work to screen for anemia. Black patients were given a mean of 10, while white patients were given a mean of 6.33. Given the data, respondents agreed that black patients get a screening two times more than white patients. An Anova on the data collected for Scenario 1, showed a F score of 145, and a p value inferior to 0.01. Post Hoc t-tests showed a significant difference between the white and black patients for question 4. For the following bias question, question 4, participants were asked if they would ask the patient about her insurance. Participants gave white patients a mean of 1.67 while black patients were given a mean of 9. Blacks were given a higher mean compared to whites. Blacks were nine times more likely asked about insurance than whites, which shows a significance in the data and shows that assumptions were possibly made about their insurance status. This correlation continues for bias questions that asked

Fig. 1 Scenario 1
Light colored bar: white patients; Dark colored bar: black patients

Fig. 2 Scenario 2
Light colored bar: white patients; Dark colored bar: black
Light colored bar: white patients
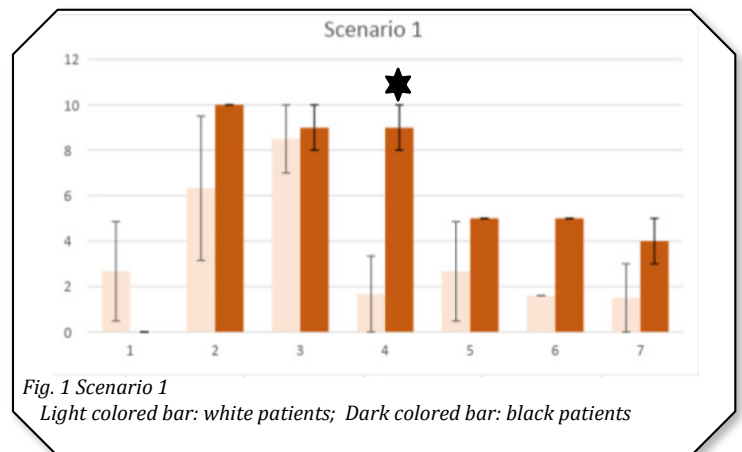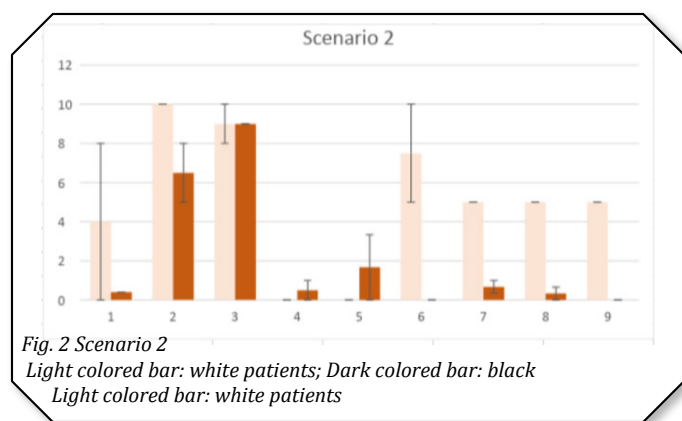
participants, about asking patients about the relationship between the father of her child, living situations and patient advice, although there was no significance detected by the t-tests, but only trends. It is important to note that the low number of responses may have distorted the data. Given the data, participants gave an average mean of 5 for black patients while white patients were given a mean of 1. Black patients are five more times likely to be asked about these personal questions which might interfere with their care given more than white patients. These questions may reflect assumptions that black patients may not have insurance, or may not receive adequate family support.

Scenario 2

For the second case study, participants are given a scenario of a mother who has preeclampsia. The patient complains of extreme nausea, weight gain, headaches, and abdominal pain. The mother explains that she has not urinated in a long period of time and that her vision is blurry. The mother's blood pressure is 140/90. Participants were asked if they would the patient if she's been stressed lately. Participants gave a mean of 0.4 to black patients while white patients received a mean of 4. In this situation, whites were four times more likely to be asked if they are stressed. The results show that whites are assumed to be more professional and thus have a higher amount of stress than blacks. Given the data, participants had a better understanding of a white person's level stress than a black patient because white people seemingly have a higher occupation compared to black.

Participants were asked if they would check the patient's blood pressure. Participants gave black patients a mean of 6.5 while white patients were given a mean of 10. White patients were given a higher mean indicating that whites are two times more likely to get their blood pressure re-checked while black patients are not. Both races had equal outcomes as the participants were asked to run a urinalysis check to see if there is excess protein in the mother's urine. Participants gave white patients a higher mean when it comes to getting information about insurance, relationship, and living conditions. Caution has to be taken not to misinterpret results since the number of participants responding to the study was so low.

Scenario 3

For the third scenario, the mother is delivering and her baby has Meconium Aspiration Syndrome. The participants are told that the patient is currently delivering and when she pushes the fetal heartbeat is slowing down and the patient's blood pressure is increasing. The amniotic fluid is brownish-green. The participants were asked if they would give the patient pain medication. Both races gave the same mean to both races. This gives the intentions that the participants would give pain medication due to the fact that she is delivering. The participants were asked if they would wait for the baby's heartbeat to return to normal before proceeding. Blacks were given a mean of 6.5 while white patients were given a mean of 10. Black patients are two times more likely to have a procedure that would make

the doctors stop treatment until the baby's heartbeat would return to normal. This is a riskier decision made for black patients. Participants were also asked if they would give patients an amnioinfusion. White patients received a mean of 2.50 while black patients received a mean of 5. Amnioinfusion isn't a treatment for MAS that is fully proven, however, blacks were 2 times more likely to receive this treatment more than white patients. For the bias questions, Black patients were two times more likely to be asked about their insurance, living conditions, and the relationship with the father of their baby more than white patients

Scenario 4

For Scenario 4, the mother is described as having a rupture cesarean as she is giving birth. The Participants are told that the patient has a long history with a previous C-section and is currently laboring during a trial vaginal birth after cesarean (VBAC). The uterine c-section scar tears open and the patient experiences severe abdominal pain. The fetal oxygen levels and heartbeat start to decrease. The fetus is expelled from the uterus and is located in the peritoneal cavity. The participants were asked if they would give the patient a large amount of medication. Blacks were given a higher mean than white concluding that there are more likely to receive a larger amount of medication in this situation. As repeated before in the previous case studies for the biased questions Black patients were three times more likely to be asked about living conditions, insurance and the relationship with the father than whites.

Personal Bias

Fig. 3 Scenario 3
Light colored bar: white patients; Dark colored bar: black patients

Fig 4 Scenario 4
Light colored bar: white patients ; Dark colored bar: black patients
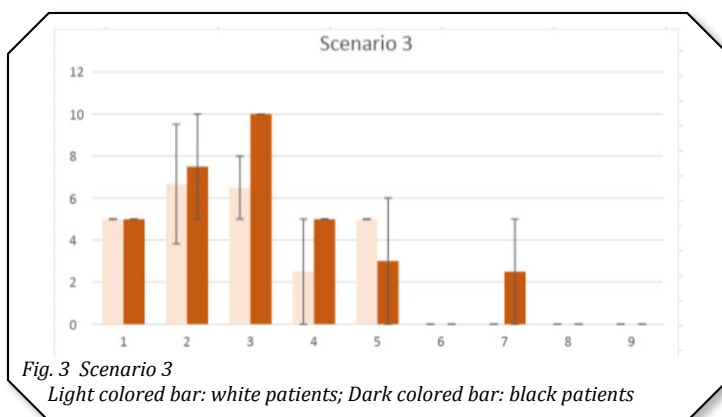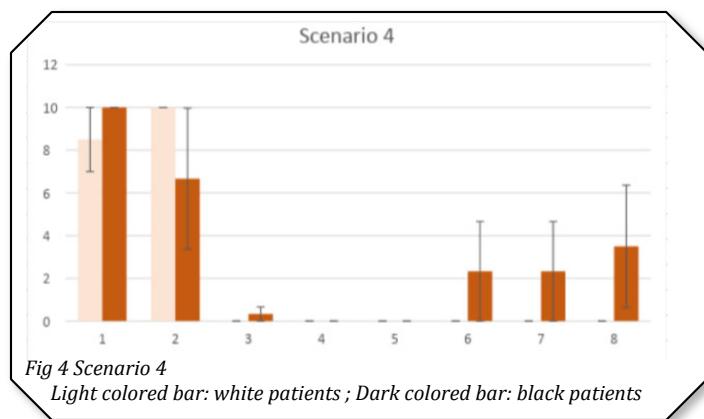
The survey was given to the participants also had a section that would test their personal bias. This section of the survey provided five personal bias questions that the participant might have. In this section the answers derive from the Likert scale, ranging and answer of 1 meaning that they disagree to 10 that they agree. This data was analyzed together with all of the participants from each survey in a small population. The first question asked the participants that if they are aware of their own subtle expression of race bias. All of the participants gave a mean of 4, indicating that almost all of the participants are not aware of their racial bias when it comes to treating patients. Participants also gave a mean of 5 when they were asked if they are aware of their racial bias in their work environment. This truly indicates that the participants are not aware of racial bias in their work environment. The participants (physicians or medical students) gave a mean of 6 when asked if race bias was an important problem affecting clinical care, and also if they think that race bias is an important contributor to a negative work life. However, the participants do express that they are motivated to eliminate the influence of stereotypes, with a mean of 8.

Action on Regular Bias

An Action on Regular Basis Test was added to the surveys; it asked participants to recognize racial bias in their daily routines with patient-interactions. This test was given to see if participants felt confident or risky interactions that had to deal with exposing racial bias and if they felt confident that their institution would address bias and had the mechanisms to address it. Participants are somewhat confident about recognizing race bias during interactions, making decisions based on racial stereotypes, speaking about racial bias in a clinical encounter, and taking action if they see a microaggression towards another race. Participants are very confident in replacing information that challenges racial stereotypes in a clinical encounter, adopt a perspective of a colleague of a different race, and challenge a work-related decision if it's been influenced by race bias. However, participants are not confident in speaking about a racial bias in a work-related interaction. Participants will not engage in taking action if they observe a microaggression toward another patient, speaking about a racial bias in a clinical encounter and work interaction. But, participants might engage in replacing information that challenges racial stereotypes in a clinical encounter, recognizing racial bias in a work and clinical encounter.

Concern Scale

The concern scale was five questions, asking participants their feelings about some aspects that contribute to racial bias. First, the participants were asked if they are personally concerned about race discrimination. Participants gave the impression that they were not concerned with racial discrimination as they gave a mean of 5.88 out of 10 on the Likert scale. Then participants were asked if they believe that people need to stop focusing so much time and energy worrying about race discrimination. People believe that racial discrimination is an important issue as people gave a mean of 1.66; indicating that they disagree with the statement. When the participants were asked about their feelings toward black and white Americans, the data showed that participants gave a mean of 6.85 and 5.88.

Discussion

Due to the limited number of the responses, it did not allow for any valid statistical tests, except for scenario 1, thus, there were only trends that could be made. These trends are clear but they can't indicate racial bias in the health care system for an entire population. Only Scenario 1 yields some statistics that show an overall significance. However, the other three scenarios cannot be computed; the statistics are limited due to the small sample size, but trends in the data are observed to make some correlations. For the demographics of the survey, it shows that the participants were 78% White, 11% Black, and 11% Asian. The ratio balance is tilted more to White Americans, which might affect the statistics with a bias. 89% of the participants were medical students in their third year, while 11% of the participants were in their 4th. However, none of the participants were physicians, which might show a discrepancy in the data as medical students might not be fully comfortable expressing racial bias in their medical institution due to possible consequences on their education. There were participants that started the survey but, stopped answering questions when they were asked about racial bias questions. The reluctance of answering bias questions could show that some of the participants that are involved in the health care system are not comfortable with discussing any implicit racial bias that they might have.

Despite the small number of participants in the surveys, there were some trends that were visible. Participants showed that white patients are more likely to be put on bed rest and are four times more likely to be asked if they are stressed. Whites are assumed to be more professional and thus have a higher amount of stress than black. The focus of the patient needs is being overlooked due to this assumption. Whites are also two times more likely to get their blood pressure re-checked while black patients are not. This shows

that white patients are more likely to receive care that requires caution in the near future. However, black patients receive care that is riskier. According to scenario 3, black patients were two times more likely to receive treatment that is not fully proven that it will work. Black patients were also two times more likely to have a risky procedure that would make the doctors stop treatment until the baby's heartbeat would return to normal. This supports the idea that vulnerable patients, elderly, mentally ill, overweight, minority and disabled, who are receiving care, are typically in groups that may be put at risk by the implicit racial bias of doctors. (FitzGerald, C., & Hurst, S. 2017). Which also emphasizes the fact that black patients are assigned less pain medication compared to white patients but are diagnosed with more diseases (Greenwald AG, et.al 2012). Black patients were nine times more likely to be asked about their insurance compared to whites. Patients who are minorities often have treatment decisions based on their insurance status (Ansell D, and McDonald E. 2015). Black patients were also more likely asked about the relationship between the father of the child and the mother, and the living conditions of their home. These personal questions can interfere with black patient care, as treatment decisions might be based on the patient's circumstances

With the few numbers of participants, the surveys revealed that almost all of the participants are not aware of their own bias and the racial bias in their work environment. Also, almost all of the participants will not engage in observing microaggression of a colleague of another race and addressing racial bias in a work-related interaction. This shows that participants might have an implicit racial bias that may affect treatment decisions, but due to the limited amount of participants, this trend may not be applicable to the entire populations of physicians in the health care system. This project is an ongoing study and that I am still accepting more responses and possible participants that are especially fully trained physicians. I hope to be able to confirm some of the trends that I have observed.

Conclusion

If physicians have a large implicit bias, then there will be a disparity in the healthcare system, increasing the infant mortality rate of black infants; because caregivers are not aware of their own bias. In my study, there is a low number of participants but there are some trends that do indicate bias, however, I need more responses to clearly indicate that indeed my hypothesis is supported. The trends in my data do support that vulnerable patients, elderly, mentally ill, overweight, minority and disabled, who are receiving care, are typically in groups that may be put at risk by the implicit racial bias of doctors. (FitzGerald, C., & Hurst, S. 2017). It also emphasizes the fact that black patients are assigned less pain medication compared to white patients but are diagnosed with more diseases (Greenwald AG; et.al 2012). Patients who are minorities often have treatment decisions based on their insurance status (Ansell D, and McDonald E. 2015). The trends may indicate that there might be implicit racial bias present as participants were answering questions, but a larger number of and a diverse pool of participants can only truly indicate this bias.

For future plans for this project, we will continue to take participants for the surveys in the meanwhile, to get a larger amount of participants. There's a possibility that the questions might be generalized and revised to reduce the length and the time necessary to take the survey. Our aim will be not to just limit the surveys to medical students but to included fully trained physicians as well. Maybe at the end of each survey, they would be an option for the participant to know if they have an implicit racial bias. In the future, we would like to attach the surveys to a training class that will be taught in a local medical school. This training class will not only teach medical students how to limit their bias while practicing, but it will bring awareness to institutions that may have racial bias in their faculty.

From the start of my study, my goal was to lower the percentage of implicit bias, in consequence decreasing the mortality rate in the healthcare system, by making physicians aware of their biases. This goal was not met, but my project has however informed some people to reflect on their own implicit racial bias. With my future plans, of expanding my project to more individuals and to

Action Questions	Mean Scores for Observing	Mean Scores for Engaging in Action
Recognize when race bias is occurring during a work-related interaction (e.g., work rounds, morning report, Grand Rounds).	3.87	3.1
Recognize when race bias is occurring during a clinical encounter.	3.8	3.1
Speak up when I observe or experience race bias in a work-related interaction (e.g., work rounds, morning report, Grand Rounds).	2.1	1.6
Speak up about race bias in a clinical encounter.	3	1.5
When I find myself making an assumption based on a racial stereotype, replace that with information that challenges the stereotype in a work-related interaction (e.g., work rounds, morning report, Grand Rounds).	3.9	3
When I find myself making an assumption based on a racial stereotype, replace that with information that challenges the stereotype in a clinical encounter.	4.1	3
Adopt the perspective of a colleague who is of a different race than me.	4.3	3.3
Challenge a work-related decision if I think it has been influenced by race bias.	4.22	3.33
Challenge a clinical decision if I think it has been negatively influenced by racial stereotypes	3.33	2.16
Take action if I observe a racial micro aggression occurring toward a colleague.	3.5	2.22
Take action if I observe a racial micro aggression occurring toward a patient.	3.33	1.75

medical schools, my goals will be definitely be met.

My project will benefit both African American women and their infants, to improve the healthcare system by making physicians aware of their implicit racial bias, allowing them time to fix it in their methods of treatment. This awareness will plant a seed for designed bias training programs for medical schools and hospitals to help institutions revise their policies concerning racial bias. This will later hopefully cause the infant mortality rate of African Americans to decrease. If physicians and medical students will become aware of their bias and will be trained to recognize it and prevent it, they will be changing their method of treating patients. With this awareness training in medical institutions, such as medical schools, future doctors will be able to recognize implicit racial bias, as it occurs, and address the problem with confidence.

With the resources of doctors, medical students and social media, my project will be able to contribute to changing how we think of race in our society. My project will help change the views of others in politics, healthcare and in everyday lives of American citizens. In my future studies, I want people to be aware that everyone has an implicit bias; changing society's view of one another and eliminating the disparities in health care system by race and ethnicity. The knowledge and awareness of implicit racial bias won't just save the way that medicine is taught in institutions, but it will also save a million of lives.

(citations available upon request)

Effects of Bacterial Metabolites on the Expression of SLC6A3, the Dopamine Transporter, in HEK Cells, by James Reilly, WESEF Paper Excerpt, Junior
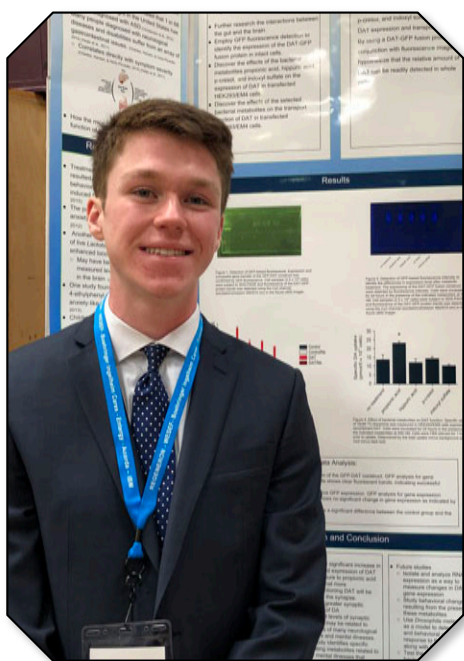
Abstract

Many mental illnesses are related to gastrointestinal disorders. Circulating metabolites produced by gut bacteria have been shown to be one possible method of communication between the gut and the brain. This study tests the effects of four circulating bacterial metabolites implicated with mental illnesses related to the gut-brain axis (propionic acid, hippuric acid, p-cresol, and indoxyl sulfate) on SLC6A3, the dopamine transporter (DAT). Based on the findings of prior studies, it was hypothesized that these four compounds would affect the expression of DAT. The plasmid pBM7-hDN containing the GFP-tagged recombinant DAT gene was used to transfect attached HEK293/EM4 cells to test the expression of DAT in the presence of the four selected metabolites. Green Fluorescent Protein (GFP) analysis verified the successful gene transfer into the cells and expression of DAT. To test expression, the transfected HEK293/EM4 cells were exposed to the metabolites at 500 µM for 24 hours. An uptake assay revealed that p-cresol increased DAT expression and propionic acid greatly increased DAT expression. On the other hand, the uptake assay also showed that hippuric acid and indoxyl sulfate decreased DAT expression. A GFP analysis showed no significant difference in fluorescence intensity, indicating no significant changes in expression after exposure to any of the metabolites. The GFP approach to verify expression and measure gene expression in this study is a major technological improvement over other methods due to its simplicity and straightforward detection method. Altered DAT expression in the brain results in an altered synaptic clearance of dopamine which is related to many mental illnesses implicated with the gut-brain axis. It was concluded that circulating bacterial metabolites do, in fact, play a major role in the casual and complex communication between the gut and the brain.

Introduction

In the world today, millions of people suffer from neurological diseases and disabilities, such as depression, anxiety, Autism Spectrum Disorder (ASD), and schizophrenia. The prevalence of these such diseases in the United States is very alarming and sparks many people's interest. A study done in 2012 estimated that 1 in 68 children aged eight in the United States has been diagnosed with ASD (Christensen et al., 2012). Along with this, many people diagnosed with ASD suffer from an array of gastrointestinal problems. One study shows that children diagnosed with ASD are three times more likely to experience symptoms such as abdominal pain, bloating, constipation, pain on stooling, sensitivity to foods, and diarrhea than the controls. The severity of these GI issues correlated with the severity of many symptoms related to ASD, including social withdrawal, irritability, and hyperactivity (Chaidez, Hansen, & Hertz-Picciotto, 2014). Other neurological disorders report gastrointestinal issues that also correlate with symptom severity, such as schizophrenia, Rett syndrome, cerebral palsy, and major depression (Heijtz et al., 2011). These findings

support the communication between the gut and brain, known as the gut-brain axis. However, how the gut and brain interact is still unknown.

In the past, studies have further supported the gut-brain axis by exposing the effect that probiotics, live bacteria or bacterial products, have on, both, the brain and behavior. For example, the implementation of Bifidobacteria treatment resulted in reduced levels of 3,4-dihydroxyphenylacetic acid (DOPAC) and decreased levels of 5-hydroxyindoleacetic acid (5-HIAA) in the amygdaloid cortex and the frontal cortex in rats, respectively (Desbonnet et al., 2010). Another study expressed how the introduction of live Lactobacillus plantarum PS128 significantly increased the levels of serotonin and dopamine in the brain. The enhanced locomotor activity observed in this study may have been caused by the increased transmission of dopamine after the probiotic treatment (Liang et al., 2015). On top of this, continuous L. helveticus NS8 treatment resulted in the reduction of biochemical, behavioral, and cognitive abnormalities induced by chronic stress in adult specific pathogen-free Sprague-Dawley rats (Liang et al., 2015). Also, the probiotic Lactobacillus rhamnosus was able to modulate behavior in mice by reducing activity related to anxiety. This probiotic also decreased the augmentation of plasma corticosterone levels in mice induced by stress (Cryan & Dinan 2012). All of these findings support communication between the gut and the brain along the gut-brain axis. The consistent demonstrations of probiotics altering brain and behavior provide insight into how our gut and brain interact. More importantly, the results of these studies expose the possible roles probiotic administration may play in controlling and manipulating the gut microbiota, the brain, and the interactions between them.

Bacterial metabolites are substances that act as intermediates and endpoints of biological processes, making

metabolites essential to proper function of the body. Metabolites have been shown to enter the brain, which may be one method of communication between the gut and the brain (Hsiao et al., 2013). For example, propionic acid, a stomachic metabolite produced by bacteria, has been shown to access the brain, both passively and actively, by crossing the gut-blood barrier and the blood-brain barrier (Thomas et al., 2012; Conn et al., 1983). The metabolite 4-ethylphenyl sulfate (4EPS) is one metabolite that has been shown to influence behavior. 4EPS is of particular interest due to the potential role it plays in behaviors relevant to ASD. Anxiety-like behavior was observed after naive mice were treated with 4EPS potassium salt from 3 weeks to 6 weeks of age. This behavior of the mice treated with 4EPS was similar to the observed behavior in the offspring of mice injected with the viral mimic poly (I:C) during pregnancy in order to activate the immune system. The offspring of these Maternal Immune Activation (MIA) mice exhibit many behavioral symptoms relevant to ASD. These results suggest that metabolites may cause or influence symptoms associated with ASD and other neurodevelopmental disorders (Hsiao et al., 2013). Additionally, many studies have demonstrated how metabolites have the ability to alter the production of neurotransmitters. One study introduced metabolites produced by spore-forming bacteria, such as 4-aminobenzoic acid (PABA), α-tocopherol, and tyramine, to germ-free mice. After the metabolites were introduced, there was an increase in the biosynthesis of 5-hydroxytryptamine (5-HT, serotonin) in specialized endocrine cells, called enterochromaffin cells, in the gastrointestinal tract. It was discovered that these same metabolites increased the expression of tryptophan hydroxylase 1 (TPH1), implying that the metabolites communicate with enterochromaffin cells, signaling the enhancement of 5-HT biosynthesis (Yano et al., 2015). Even the bacteria

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that produce these metabolites have been shown to influence neurotransmitter production. The absence of the native microbiota that produces these metabolites disrupted the levels of serotonin in the hippocampus, suggesting that the metabolites affect related neural process (Clarke et al., 2013).

Children with ASD have been shown to have some abnormal levels of metabolites mostly due to the overpopulation of Clostridium species in the gut. After analyzing the urine of 62 children diagnosed with ASD and the urine of 62 non-ASD children, all 1.5-7 years of age, it was found that the urine of the children diagnosed with ASD had significantly higher levels of the compounds 3-(hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), 3-hydroxyphenylacetic acid (3HPA), and 3-hydroxyhippuric acid (3HHA) than in the controls. After the administration of oral vancomycin treatment to children with ASD, their urinary levels of
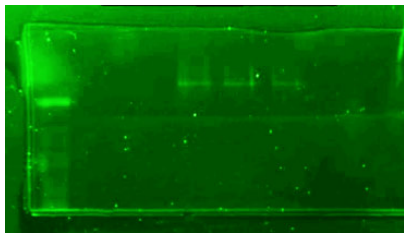
Fig. 2. Expression of DAT in transfected HEK293/EM4 cells through GFP visualization

HPHPA, 3HPA, and 3HHA decreased dramatically, indicating that these metabolites may be produced by the Clostridium species in the gut (Xiong, Liu, Wang, Zeng, & Peng, 2016). The metabolite HPHPA is of notable interest due to its ability to inhibit dopamine beta hydroxylase, a compound required during the process of converting dopamine into norepinephrine. The inhibition of dopamine beta hydroxylase may be related to excess amounts of dopamine associated with schizophrenia and psychotic behavior (Shaw, 2010). The influence that bacterial metabolites have on behavior and brain function suggests that these compounds may be the path of interaction between the gut and the brain.

Metabolites have been found to influence the brain directly. Propionic acid is one such metabolite to do so. Upon entering the brain by crossing the blood-brain barrier, a barrier that tightly regulates the movement of molecules between the blood and the brain (Daneman & Prat, 2015), through the use of high affinity transporters, studies have shown this metabolite affecting an array of neurological functions, including the release of neurotransmitters, mitochondrial functions, and gene expression (Thomas et al., 2012; Conn et al., 1983; DeCastro et al., 2005; Maurer et al., 2004). Propionic acid has been shown to alter the release of neurotransmitters, including serotonin and dopamine after entering the brain (El-Ansary, Bacha, & Kotb, 2012). These results of the ability of propionic acid to cross the highly restrictive blood-brain barrier and affect neurotransmitter release expose the possibility of neurotransmitter signaling to be affected by metabolites. One study conducted to discover the effects of antibiotic treatment on the brain also measured the change in expression of neural signaling-related molecules. After antibiotic treatment, the metabolites propionate, p-cresyl, trimethylamine-N-oxide, and deoxycholic acid were recorded at dramatically altered levels. This study tested the expression of different neurotransmitter transporters after antibiotic treatment. Neurotransmitter transporters bind to neurotransmitters in the synapse between neurons, terminating neurotransmission by releasing the bound neurotransmitter back into the presynaptic cell (Rudnick, 2002). Among the tested transporters in the brain was SLC6A4, the serotonin transporter. After the antibiotic treatment, the expression of SLC6A4 mRNA in the hypothalamus and hippocampus was slightly reduced when compared the vehicle-treated mice. On the other hand, the expression of mRNA was increased in the medial prefrontal cortex and greatly increased in the amygdala (Frohlich et al., 2016). A more recent study tested the function and expression of SLC6A3, the dopamine transporter (DAT), after exposure to metabolites. After infecting HEK293-EM4 cells with a recombinant bacmid containing the recombinant dopamine transporter gene, an assessment of the expression and function of DAT as a result of exposure to the metabolites propionic acid, indoxyl sulfate, hippuric acid, and p-cresol was performed. By conducting a bicinchoninic acid assay, western blot, and uptake assay, it was revealed that exposure to these metabolites resulted in altered expression of DAT. It was concluded that the exposure of p-cresol and propionic acid increased the expression of DAT, while indoxyl sulfate and hippuric acid reduced the expression of DAT (Chung, 2017). The ability of metabolites to affect the neurological processes of neurotransmitter release and reuptake sparks the speculation whether or not metabolites play a role in the gut-brain axis.

In many studies, gene expression has been detected through complex approaches such as RNA isolation and analysis, western blots, and uptake assays. However, recently, alternative methods have been proposed. Green fluorescent protein (GFP) is a proposed way that gene expression could be detected. One study reported that GFP is a reliable reporter of gene expression and the intensity of GFP fluorescence is directly proportional to GFP mRNA abundance in cells (Soboleski, Oaks, & Halford, 2005). Another study verified that a GFP reporter system is able to effectively assess successful gene transfer and expression in human hematopoietic progenitor cells (Cheng et al., 1997). GFP analysis has been supported by the traditional methods of gene expression analysis but is a much more simple and efficient approach.

Statement of Purpose

Previous studies have found that bacterial metabolites may play a very important role in the communication between the gut and brain. Many people who suffer from mental illnesses and neurological disorders show an altered profile of bacterial metabolite levels. Probiotics have been shown to alter levels of dopamine and serotonin in the
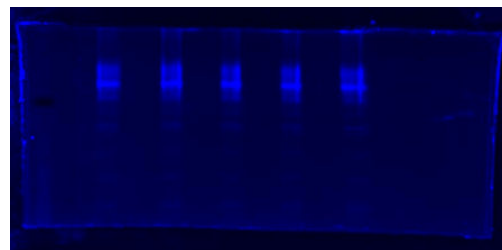

Fig. 3. Expression of DAT in the presence of the selected metabolites at a concentration of 500 nM was measured using GFP visualization. The order from left to right was no treatment, propionic acid, hippuric acid, p-cresol, indoxyl sulfate, control cells (no DAT). The intensity of each band indicates the expression of DAT for each condition.

brain, providing evidence that supports the fact that monoamines and their transporters are implicated with the interaction between the gut and the brain. On top of this, altered levels of circulating metabolites caused by antibiotics induced cognitive impairment caused by a change in expression of the serotonin transporter. Additionally, bacterial metabolites have the ability to affect the expression of the dopamine transporter at a molecular level. In order to further investigate these findings, it was hypothesized that metabolites that have implications with a variety of mental illnesses could also affect the expression of DAT mRNA. The four metabolites selected for this study were propionic acid, p-cresol, hippuric acid, and indoxyl sulfate. Uptake of ^{14}C-dopamine and Green Fluorescent Protein (GFP) visualization was performed to measure the function and expression of DAT in attached HEK293/EM4 cells in the presence or absence of these compounds.

(...)

Results

The successful transient transfection of the plasmid pBM7-hDN and expression of DAT in the HEK293/EM4 cells was confirmed using GFP analysis. The green fluorescent bands seen in Fig. 2 indicated the presence of the GFP-tagged DAT in the transfected cells. The intensity of GFP fluorescence under ultraviolet light has been shown to be directly proportional to gene expression and protein concentration on eukaryotic cells (Soboleski, Oaks, & Halford, 2005) Fig. 3 shows that the expression of DAT in the presence of the selected metabolites was not significantly different than the expression of DAT in the control group. All of the bands are equal in fluorescence intensity, indicating that the expression of DAT was not altered by the metabolites.

An uptake assay was performed to test the effects of the metabolites on the expression of the dopamine transporter. The experiment tested the expression of DAT in the presence of the selected metabolites at 500 nM after 24 hours of

exposure. In Fig. 5, non-transfected cells (no DAT) were used for the negative control group in the uptake assay, as seen by the black bars. The positive control group consisted of transfected cells expressing DAT that were not exposed to the metabolites. To ensure that DA

Fig. 4. This figure shows the control group (no DAT) in the black bars, the control/nisoxetine group (no DAT) in the open bars, uptake of 10 uM 14C-dopamine in the presence of each metabolite at 500 uM in the bright red bars, and the non-DAT specific binding in the dark red bars.

binding is DAT specific, non-specific binding was determined with 1 μM nisoxetine. Nisoxetine is a DAT blocker. Any DA uptake that takes place in the presence of nisoxetine is not through the dopamine transporter, so this binding is non-specific. General uptake is represented by the bright red bars while non-specific binding is represented by the dark red bars.

Fig. 5 shows the DAT-specific binding of DA in the performed uptake assay. This figure was formed by taking the difference between the bright red bars (general uptake) and their respective dark red bars (non-specific uptake) from Fig. 4. Uptake was greatly increased in the presence of propionic acid at 500 nM and slightly increased in the presence of p-cresol at 500 nM. On the other hand, uptake was slightly decreased in the presence of hippuric acid and indoxyl sulfate. The increased uptake indicated that more DAT was expressed and present to bind with the dopamine. The decreased uptake indicated that less DAT was expressed and present to bind with the dopamine.

Discussion and Conclusion

In a previous study, gut dysbiosis induced by antibiotics altered the profile of circulating bacterial metabolites in mice (Frohlich et al., 2016). Some of the

metabolites altered in the previous study were similar to the metabolites used in this study, including propionic acid and p-cresol. The previous study reached the conclusion that gut dysbiosis caused by antibiotics can alter the expression of SLC6A3, the serotonin transporter (Frohlich et al., 2016). However, this study tested the effects of bacterial metabolites on the expression of SLC6A3, the dopamine transporter. The altered uptake of ^{14}C-dopamine after exposure to the selected metabolites indicates that circulating bacterial metabolites can, in fact, affect the expression of the dopamine transporter. Altered expression of DAT is related to a variety of mental illnesses implicated with the gut-brain axis. The increase in expression of the dopamine transporter by propionic acid and p-cresol shows that more DAT would be present in the brain. An increase of DAT in the brain causes greater synaptic clearance of dopamine by the presynaptic neuron. The decreased
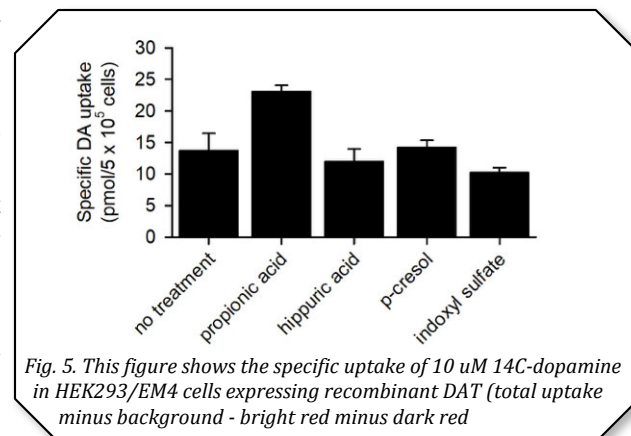

Fig. 5. This figure shows the specific uptake of 10 uM 14C-dopamine in HEK293/EM4 cells expressing recombinant DAT (total uptake minus background - bright red minus dark red

levels of synaptic dopamine may be related to the symptoms of Autism Spectrum Disorder, anxiety, depression, and other mental illnesses related to the gut-brain axis, such as schizophrenia. The causal relationship between the expression of DAT and many mental illnesses implicated to the gut-brain axis proves that circulating bacterial metabolites may cause or influence these mental illnesses.

This study has many technological improvements since the prior studies. The study on which this project is based used uptake methods to verify the expression of DAT in HEK293/EM4 cells and a western blot to measure the expression of DAT after exposure to the selected metabolites. In this study, the use of a GFP-tagged DAT construct is entirely new. Functionality has been shown in the quick uptake of dopamine. The GFP-tagged version is new, active, and can be visualized without complex labeling approaches. The GFP approach to measuring the expression of DAT is a much more simple and straightforward method when compared to other approaches, such as a western blot or RNA isolation and analysis. The visualization of GFP-tagged DAT in a low number of cells very clearly on an SDS gel is unique and a major scientific improvement.

In the future, I would like to replicate my results and look into behavioral changes resulting from the presence of these metabolites. Using Drosophila as a model to detect locomotor and behavioral changes in response to the metabolites along with DAT mRNA analysis would be very powerful and provide insight into the effects of metabolites on behavior and DAT expression at a molecular level. Future studies could also test these metabolites with other neural-signaling molecules, including the serotonin transporter, N-methyl-D-aspartate receptor subunit GRIN2B, and the brain-derived neurotrophic factor.

My study identifies specific circulating bacterial metabolites related to many mental illnesses that influence the expression of SLC6A3, the dopamine transporter. Identification of these metabolites reveals the ability of probiotic and antibiotics to treat mental illnesses relevant to the gut-brain axis. Using probiotics and antibiotics to alter the gut microbiota to one that produces bacterial metabolites that have the ability to affect neurotransmission provides a safer and more natural way to normalize monoaminergic signaling and to treat mental illnesses.

These findings of the effects of circulating bacterial metabolites on DAT expression provide a deeper insight into the casual relationship between the gut and the brain at a molecular level.

(Citations available upon request)

The Applications of Nanotechnologies in Cancer Therapeutics and Investigation: From Diagnosis to Treatment, by Paul Williams, WESEF Paper Excerpt, Junior
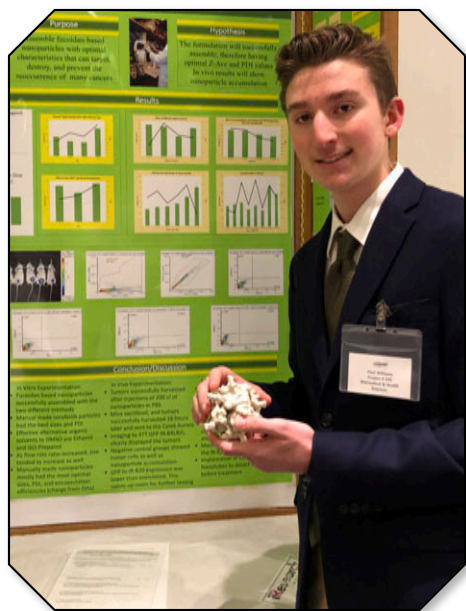
Abstract

Nanotechnologies have been common for many years and have served a wide variety of purposes, but it has mainly been within the last decade in which they gained popularity for utilization for cancer detection, treatment, and investigation. They come with a great appeal due to their potential to revolutionize modern cancer treatment by making it more cost-efficient, effective, and more bearable for patients due to them only targeting cancerous masses with accuracy and precision, as well as medical professionals having a greater control of drug dosages. A setback, however, has been the enhanced permeability and retention (EPR) effect not demonstrating enough benefit in killing cancers, though not enough proof of its occurrence. In this study, fucoidan is utilized as a contrast agent in sorafenib and trametinib nanoparticles due to its affinity to P-Selectin, which is a protein upregulated in tumor vasculature. The traditional method and a new technologically-based method of manufacturing nanoparticles are assessed to determine which synthesizes the most ideal nanoparticles for an in vivo mouse model where the EPR effect is proven due to this new formulation. The nanoparticles are characterized in vitro for the purpose of gaining a further knowledge of essential properties such as size and polydispersity index that play a key role in its stability. With this further knowledge, nanotechnologists can be one step closer in manufacturing an effective and efficient cancer treatment, nonspecific to one type of cancer.

Introduction

The field of nanomedicine includes a very broad range of studies that seem to formulate new topics based off of each other. What they have in common is that they work with nano-sized structures referred to as nanoparticles typically ranging from 1-100 nm in size to serve many functions as well as topics of study. Many of these studies include but are not limited to, breaking up clusters of bacteria to synthesize treatment, usage for treatment of cardiovascular disease such as atherosclerosis, to serve as an antioxidant to repair damage in the bloodstream or rest of body, increase growth for essential parts of the body,

and even cleaning up parts of the environment (Boysen, 2007). Seemingly enough, relatively recently all of these uses came together to treat a disease affecting many millions of people all around the world and also being one of the largest causes of death in the world, which is most commonly referred to as cancer. Tumor targeting nanoparticles function to destroy these large cancerous clusters as well as repair their damage that they have done to healthy body cells. The realization that these nanoparticles are known to accumulate in tumor sites is expressed in the phenomenon of the EPR, or enhanced permeability and retention effect. When engineering a nanoparticle, this phenomenon heavily influences what the nanoparticle is composed of, as well as simply the tumor targeting process itself. Upon the synthesis process of the nanoparticle through the bloodstream, it will accumulate and recognize that a cell is cancerous or abnormally growing through it settling in gaps existing between cells in the tumor. This is a reliable feedback system that makes tumor targeting very reliable. The buildup of nanoparticles in the tumor site is the main goal of treatment, leading to the destruction of the tumor (Greish, 2010). This can be donein several different ways, which depend ultimately on the structure and composition of the nanoparticle. Two main methods that have been studied and tested are through thermal ablation,

and cellular apoptosis (Cormode et al., 2009). Thermal ablation is the method nanoparticles utilize to heat to solid cancerous mass to such a high temperature where it cannot thrive and function any longer leading to its demise. Cellular apoptosis, however, a more natural approach, introduces cytotoxic or "toxic to living cells" agents to the tumor which cause it to naturally commit suicide (Gianella et al., 2011). This may seem ideal that nanoparticles can just perform just simply these functions, but they also aid in reversing the damage due to the tumor along the way to the cancer site, or most likely at the site due to the EPR Effect. When a cancer cell is formed somewhere in the body, it's the main function is to grow and obtain more nutrients. This is done through the process of angiogenesis, which in part of its process secretes VEGF or Vascular Endothelial Growth Factor protein. Angiogenesis is formally defined as the formation of new cells from pre-existing ones. The VEGF protein is secreted as the "signal protein" to stimulate the formation of the new cells which ultimately are created to be sent to the tumor as nourishment for it to grow and flourish. Under ideal circumstances, a tumor can thrive for years and even spread. Abnormal cell growth, angiogenesis and the spreading of tumors is why cancer is so fatal, and often is not dedicated to one location. The synthesis of nanoparticles and the creation of

nanoemulsion platforms work to neutralize the negative effects of the VEGF protein and show a promising future for cancer research. (Gianella et al., 2011).

Most cancer treatments available currently for serious cancer diagnoses are relatively ineffective, due to them often having more negative effects than positive results. Treatment therapies such as chemotherapy, and radiation therapy, introduce very strong toxins in high dosesto the body, and most of the time the patient suffers more bodily damage from the traditional devices, than from therapeutic agents and even cancer itself (Zhu et al., 2014). The immune system is also heavily affected by these treatments, weakening it, and also making it more susceptible to many types of pathogens while on treatment. Nanomedicine for the purpose of tumor targeting works to exclude most harm from treatment, and just focus on small doses. There are other key benefits attributed to tumor targeting nanoparticles. Cancer has the highest probability of being overcome when it is detected as early as possible. This is so because, at the earliest stages of development of the tumor, it is the least nourished, and shows the most signs of being an abnormally growing mass. Nanoparticles with the use of certain imaging techniques as well can detect and diagnose cancer much quicker than most modern cancer treatments (Zhu et al., 2014). The precision of nanoparticle tumor targeting treatment is unmatched

Figures 1 and 2: blue represents size, orange represents PDI
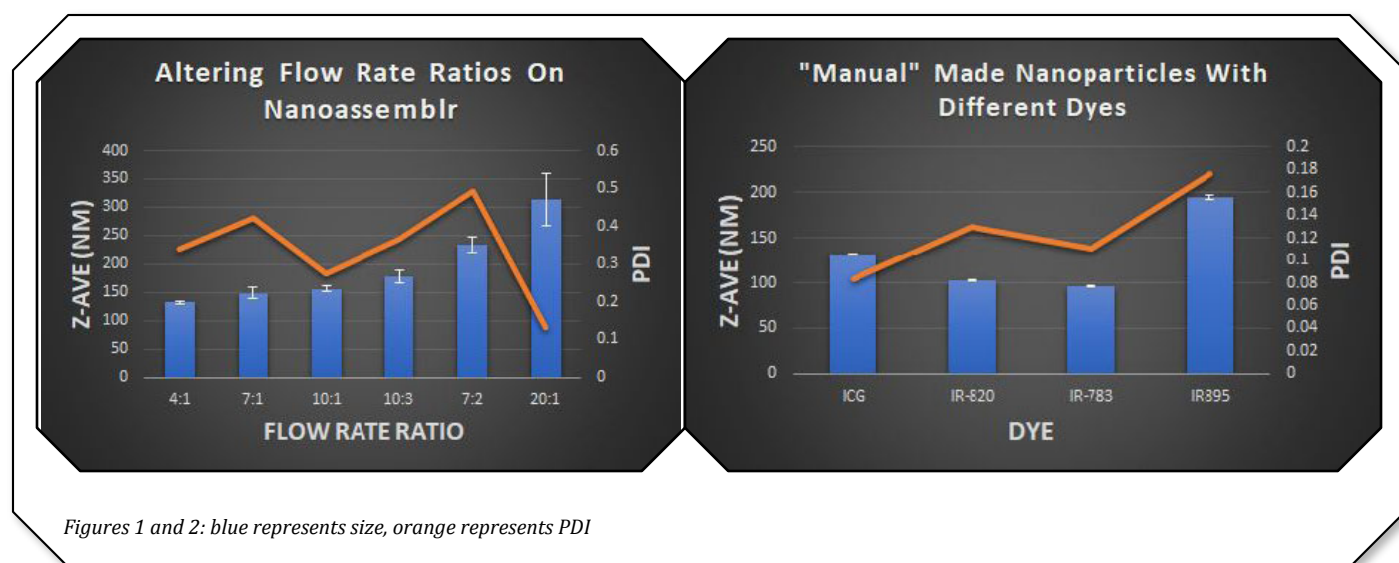
for repairing tissue deep in the body, anywhere in it, and for the destruction of the tumor mass. Mostly beneficial in terms of cost, the synthesis and engineering of tumor targeting nanoparticles are much cheaper than most treatments available today, due to them being designed with natural and cost efficient components such as iron oxide. They perform better at tumor targeting therapy than treatments most commonly used today (Zhu et al., 2014). As aforementioned, tumor targeting nanoparticles only work for the destruction of cancerous tumor masses and not healthy body cells. All these factors, are why research of tumor targeting nanoparticles have become so popular within the past decade.

Nanoparticles are man-made, mainly engineered with natural components. Materials such as iron oxide and quantum dots serve as a natural basis for the structure of them (Jarzyna, P. A., Skajaa, T et al,. 2009). They can also be referred to as a micelle that has a cytotoxic cancer drug encapsulated in to deliver to the tumor (Jarzyna, P. A., Gianella, A., et al., 2011). This is known as nanoparticle drug-delivery and is a concept that has been studied in much depth over the past decade, and is a recurring concept in most review and research papers. This is so due to the fact that the body's immune system doesn't attack the nanoparticles as they circulate through the bloodstream, and reacts to them as they are a normal body cell. With this fact known, cancer drugs can

now be delivered directly to the tumor site, and treat it a known and controlled dosage to make therapy as less toxic to the whole body as possible. The outer portion of the nanoparticle, hydrophilic, and the interior or encapsulated drug hydrophobic is specific to these qualities in order to get them through the bloodstream which is also an aqueous environment (Cormode et al., 2009). Most of the outer and middle layers of the nanoparticles assist in the destruction of the tumor mass through processes such as thermal ablation or cellular apoptosis and assist in imaging-guided therapy for the nanoparticle. At this time these studies are still experimental and have not been expanded or implemented on a larger scale.

In previous experimentation, polymer-based nanoparticles were made, then the self-assembly mechanism was utilized to form these materials into the nanoparticle shape. The most common contrast agents in these experiments were iron oxide and gold, for the purpose of tracking the location and progress of in vivo nanoparticles with imaging techniques such as Magnetic Resonance Imaging, Near-Infrared Fluorescence Imaging, and transmission electron microscopy (Mieszawska et al., 2013). These experiments were performed in vitro and in vivo with relative success. The nanoparticles were successfully self-assembled with an ideal size and polydispersity and in vitro imaging

techniques were successful in providing essential information to characterize them enough for the researchers to become educated about them enough to be applied to an in vivo model. In the experiments, the in vivo models most often were nude adult mice with colon cancer along with a control group that did not receive any treatment. The nanoparticles were efficient in shrinking the size of the tumor in the experimental group, but there were several limitations from the experiments. The most major one and the main focus of this study was that there was no way of proving the enhanced permeability and retention effect with this nanoemulsion platform. There was no way of knowing if all the nanoparticles accumulated at the tumor site without reaching any other cells. This research gap is what makes many skeptical about nanomedicine along with the enhanced permeability and retention effect.

In order to prove the enhanced permeability and retention effect, it must be ensured that all the nanoparticles in the emulsion platform will reach the tumor site. For this to occur, the nanoparticles utilized must have a component of it that has a nanomolar affinity to the cancerous mass, and the nanoparticles should be of a size from around 75-150 nm, as well as have a Polydispersity Index (PDI) below 0.300. Not only would this increase the likeliness of all nanoparticles reaching cancer, but it can
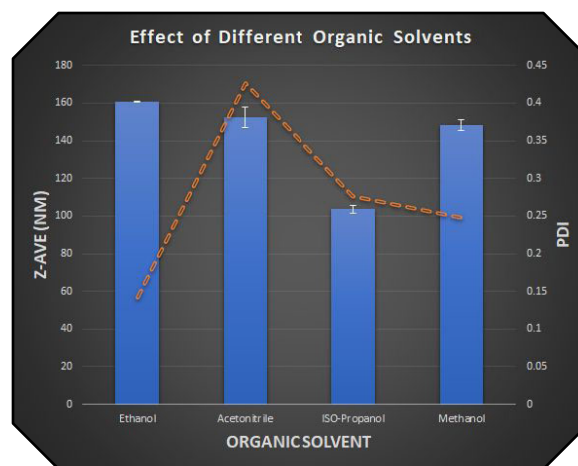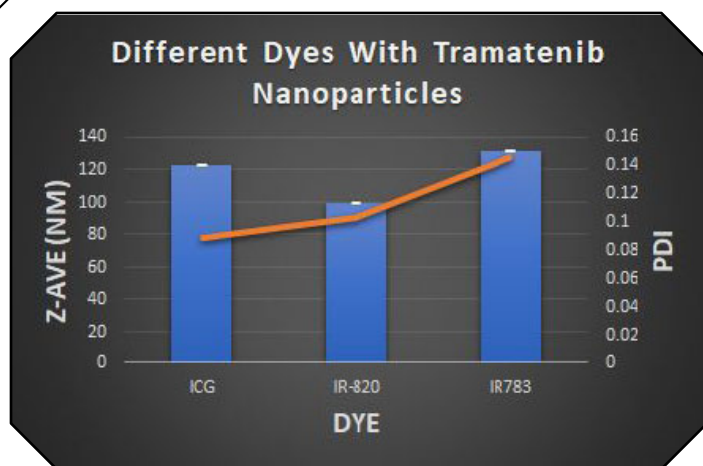
Figures 3 and 4: blue represents size, orange represents PDI

be ensured that the nanoparticles will remain stable both in vitro and in vivo. To accomplish this, this study utilized fucoidan (Fi) which has a nanomolar affinity to P-Selectin: "a molecule expressed on activated vasculature that facilitates metastasis by arresting tumor cells at the endothelium" (Shamay, Y., Elkabets, M., et al., 2016). Fucoidan is a sulfated polysaccharide with anti-tumor properties, found in many types of algae, that is very abundant, natural, and has the nanomolar affinity to P-Selectin. Through 420 clinical samples, it was found that P-Selectin is expressed in many types of human cancers including Lymphoma, Ovarian cancer, lung cancer, breast cancer, melanoma, liver, bladder, and cervical cancer. Since P-Selectin is so prevalent in the majority of cancerous masses, it became a novel idea to replace the polymer structure of most popular nanoparticle formulations with fucoidan as the main contrast agent (Shamay, Y., Shah, J., et al., 2018). In this study, fucoidan based nanoparticles, encapsulated with sorafenib (a multikinase inhibitor), along with trametinib, (a MEK inhibitor) are dissolved in an organic solvent, circulated around with an aqueous group containing the fucoidan, sodium bicarbonate, DDW, and dyes including, ICG, IR-820, IR-783, and IR-895, are synthesized, and tested in vitro as well as in vivo, in eight-week-old, female, albino mice.

Statement of Purpose:

The purpose of this experiment is to prove the enhanced permeability and retention effect by synthesizing fucoidan-based nanoparticles encapsulated with sorafenib and trametinib, with ideal sizes being anywhere from 75-150 nm, having a low polydispersity index, in order to have the most ideal nanoparticles be tested and characterized in vitro, as well utilized in vivo. Throughout the experiment, a goal is also to determine whether the time has an effect on the stability of the nanoparticle formulations, as well as to assess whether making the nanoparticles "manually" or by the "NanoAssemblr" device. In the long term, the goal of this experimentation is to be confident enough in these nanoparticle formulations to produce stable and effective nanoparticles, to be utilized for treatment of a wide variety of human cancers to provide patients better therapy with limited side effects. (...)

Results :

For the in vitro experiments, the nanoparticles synthesized were stable, and for the most part, had ideal sizes and PDIs. On the nanoassemblr, as flow rate ratio increased, size tended to as well (Figure 1). PDI tended to vary from sample to sample, but the average PDI in that experiment was about 0.250, which signifies that they are likely stable. As the experiment was run with different dyes (Figure 2) and testing the effect of centrifugation and dialysis (Figure 5), the 4:1 ratio proved to be effective in that the sizes of the nanoparticles were about 100 nm, and the PDIs were about 0.125, with some being below 0.100 which signifies they are very stable. Centrifugation was proven to be the most effective method of ensuring the stability of the nanoparticles after synthesis because the size and PDI were more optimal than those without it, or dialysis being done to the samples. Other than dimethyl sulfoxide, Ethanol, and Isopropanol were proven to be other useful options as an organic solvent to dissolve the drug in for the organic group because of how stable their nanoparticles were (Figure 4). As for the manually made nanoparticles, the trametinib ones had more optimal characteristics than the sorafenib nanoparticles (Figure 3). Overall, however, it was ironic to note that the manually made particles possessed more optimal characteristics than those made from the nanoassemblr (Figure 6). This is so because the manual method is so operator dependent, leaving very limited control over size and PDI.

For the in vivo experiments, the control

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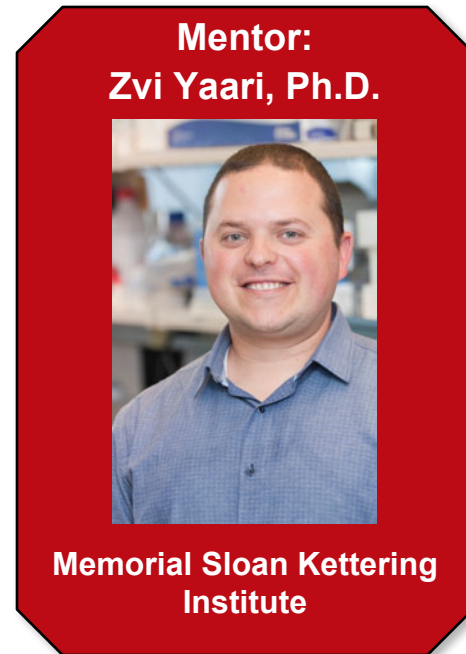
groups with no no nanoparticles injected, or just GFP, there was growth in the diameter of the tumor. When processed by the Aurora, these samples still possessed all growth factor proteins that they originally had. For the experimental group, however, the dyes of these nanoparticles were located in the tumors, which signifies the sorafenib served its function in inhibiting kinase growth factor proteins that make the tumor larger, and more nourished making them smaller in diameter (size).

Discussion/Conclusion:

Overall, this experimentation served its purpose of providing a further knowledge on the characteristics of fucoidan-based sorafenib and trametinib nanoparticles made both manually, as well as by a specialized machine which has a chip that recognizes the aqueous and organic groups and surrounds the drug around an aqueous group with precision and accuracy in order to obtain and manufacture the most precise nanoparticles possible. Although more efficient and consistent, the manual made nanoparticles possessed more optimal sizes, as well as lower PDIs which is why they were utilized for the in vivo experiments. The ability to become more familiar and characterize these nanomaterials enough to ensure their stability in an in vivo model is a
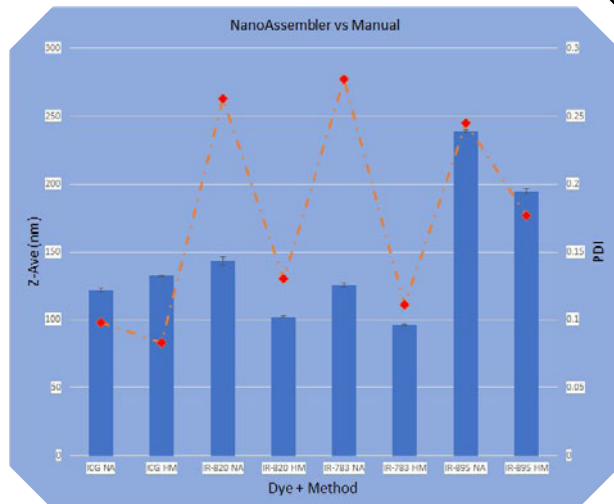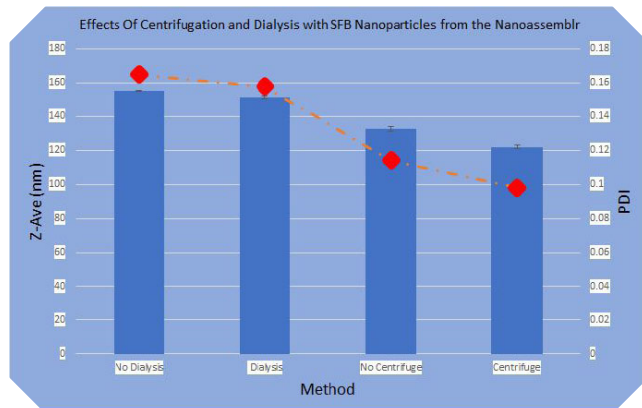
Figures 5 and 6: blue and red represents size and PDI respiecively

step closer to revolutionizing cancer therapeutics as we know it. In the in vivo experiment, the controversial phenomenon of the EPR effect was proven by a more reliable method of tumor targeting; affinity rather than only depending on size. Fucoidan is such an effective contrast agent because not only does it have the affinity for P-Selectin, but it also possesses anti-tumor properties, as well as being more natural than other polymers commonly used in nanoparticles. When traveling in the bloodstream, these nanoparticles will not target any other body cells, or even be targeted by the body's immune system which then the EPR effect, using not only the nanoparticle size to target the tumor but the fucoidan affinity to P-Selectin, which leads to the fucoidan binding to it, and the drug being released into the tumor milieu and cutting off essential growth factor proteins. This was proven by the Aurora machine expressing that the nanoparticle components were present in the tumor milieu. With a better grasp of the EPR effect, as well as a very increased knowledge of materials on the nanoscale for cancer therapeutics, comes a greater likeliness of these technologies and others such as nanosensors for the purpose of detection of cancer, to be implemented in human models to aid in treating a disease that takes the lives of millions of people worldwide, while also increasing the likeliness of providing them with a more bearable treatment as well.

(citations available upon request)

Oligodendrocytes and Multiple Sclerosis in the Central Nervous System by Adhithya Rajasekar, Junior

The nervous system uses electrical impulses to communicate with cells all over the body, which is necessary for the body to function. These electrical impulses moves through neurons, starting at the dendrites, moving through the cell body, through the axon, going across the nodes of ranvier, out the axon terminals, and across the synapse to another neuron where the process repeats. These electrical impulses are facilitated by the myelin sheath, which covers the axon.

Myelinated nerve fibers occur predominantly in the cranial and spinal nerves and compose the white matter of the brain and spinal cord. White matter refers to the areas of the nervous system that contain myelinated axons, while gray matter refers to areas in the nervous system in which the nerve fibers are unmyelinated. In unmyelinated nerves, impulses are conducted by the propagation of the action potential along the membrane of the axon. In myelinated nerves, impulses are transmitted by an slightly different process, called saltatory conduction, in which the impulse jumps from one node of ranvier to the next. Impulses in myelinated nerves are transmitted hundreds of times faster and require much less energy than in unmyelinated nerves (Miller-Keane Encyclopedia and Dictionary of Medicine, Nursing, and Allied Health, Seventh Edition).

Oligodendrocytes produce this myelin in the central nervous system. According to an article by Barateiro et al, oligodendrocytes arise from oligodendrocyte progenitor cells (OPCs) that proliferate and differentiate

just before and after birth, under a highly-regulated program. Both oligodendrocytes and their precursors are very susceptible to injury by several mechanisms, including excitotoxic damage, oxidative stress and inflammatory events (Barateiro et al. 2016).

In order to make myelin, the glial tongue of an oligodendrocyte surrounds the axon, forming a double-membrane structure which is called a mesaxon. There is an inner mesaxon and an outer mesaxon that ends in a loop, or tongue, of glial cytoplasm. This glial tongue is continuous with the plasma membrane of the oligodendroglial cell through slender processes. One glial cell can myelinate forty or more separate axons (Siegel GJ et al. 1999).

There are many disorders, both in the central and peripheral nervous system, that destroy this myelin, and these disorders can cause disastrous effects in brain function. One such disorder in the central nervous system in Multiple Sclerosis. An article by Khaled Mohamed Mohamed Koriem lists the symptoms of Multiple Sclerosis including muscle weakness, weak reflexes, muscle spasm, difficulty in movement, miss-coordination and unbalance with others. The mechanism underlying MS can be summarized into 2 reasons, " (1) the immune system destroying the myelin sheath, and (2) failure of the myelin-producing cells (oligodendrocytes) to produce new sheathes," (Koriem, 2016).

MS is considered an autoimmune disease, which is when the body's immune system attacks its own cells. The way in which this happens in MS involves T cells, which are one type of white blood cell in the immune system. They become sensitized to proteins in the CNS. It is not known what causes T cells in persons with MS to become activated, but it is postulated that both genetic and environmental factors are important, (National Multiple Sclerosis Society 2017).

The immunopathological events involved in the onset of MS can be divided into 5 steps. First is the initial T-cell priming, which occurs within systemic immune compartments and is initiated by sensitization with myelin antigens including myelin lipids. Next is activation phase of the periphery (thymus and lymph nodes), Antigens presented by antigen presenting cells (APCs) within secondary lymphoid organs induce the activation and expansion of myelin-specific T cells, and these activated myelin-reactive T cells circulate through the body searching for their specific antigens to become re-activated. Next occurs the migration of the proinflammatory T-cells across the blood-brain barrier (BBB). (Engelhardt 2006).

This is a complex multi-step process that and occurs via interactions between adhesion molecules found on the surface of lymphocytes and endothelial cells. First, circulating T cells slow in the bloodstream due to contact between distinct adhesion molecules on their surface and on endothelial cells. In the second step, homeostatic chemokines, such as CCL19 and CCL21 are produced by cells and mediate T cell activation, a step followed by third and fourth steps of firm adhesion and final transmigration of the lymphocytes. In the fifth step, CD4+T cells accumulate within enlarged perivascular spaces where they can encounter their specific antigens presented by the major histocompatibility complex (MHC) class II or CD1 on the surface of APCs such as perivascular dendritic cells. This immune synaptic contact reactivates the T cells. However, for complete activation, differentiation and clonal expansion, a co-stimulating process involving additional molecules is required. This antigen-triggered activation enables T cells to traverse the glia limitans and migrate into CNS parenchyma (Engelhardt 2006).

After this comes the amplification of local inflammation and activation of APCs, such as microglia. the autoreactive CD4+T cells initiate the local pro-inflammatory cascade.

Eventually, a variety of effector mechanisms—including antibody-mediated cytotoxicity, oxygen and nitrogen radicals, pro-inflammatory cytokines and apoptosis-mediating molecules that damage oligodendrocytes, myelin sheaths and occasionally, at this stage, axons—are induced (Becher 2006). Finally comes the effector phase of the disease, which is invasion of CNS parenchyma resulting in damaging of oligodendrocytes, myelin sheath and axons. Despite this insight into pathophysiology, the cause of MS remains unclear and definitive treatment of this frequent and chronic disease is still elusive. (Engelhardt 2006).

According to a review by Robin J.M. Franklin, disease progression is thought to be compounded from two underlying processes: myelin destruction (demyelination) with failure to remyelinate, and progressive axonal damage with little capacity for recovery (Franklin 2002). The current treatments for MS as listed in a review by Maria Podbielska, Naren L. Banik, Ewa

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Kurowska and Edward L. Hogan include, " β-interferons, IFNβ-1α (Avonex, Rebif) and IFN β-1β (Betaseron); the synthetic peptide glatiramer acetate (Copaxone); the antineoplastic agent mitoxantrone (Novantrone), and; a very late antigen-4 (VLA-4) blocker natalizumab (Tysabri)". These are only partially effective. All of these drugs are administered by injection and many MS patients prefer oral treatment. There are three new oral medications, already released and approved by the Food and Drug Administration (FDA): "fingolimod marketed by Novartis as Gilenya, dimethyl fumarate (Tecfidera, Biogen Idec, MA, USA), and teriflunomide (Aubagio from Sanofi, Paris, France)" (Podbielska et al. 2013). These drugs mainly affect lymphocyte trafficking and/or differentiation, though more needs to be done to clarify their mechanisms. These therapies aim to reduce the immune response by targeting immunological pathways. They slow down the immune response such that oligodendrocytes have time to catch up and remyelinate the damaged axons, but this treatment method isn't always effective at preventing the onset of disability. They can, however, often leave the body susceptible to infectious diseases. With the immune system slowed, foreign antigens have an easier path into the body.

A focus on remyelination may be a better path of treatment. If a method of improving remyelination can be developed, then patients can be treated without the decrease in immune capability. For that, the specific mechanisms of remyelination that are being blocked in MS need to be found. According to the study by Podbielska et al, there are a multitude of hypotheses as to why remyelination fails in MS, which may reflect either changes in environmental inputs or intrinsic pathways regulating OPCs functions. Several factors are likely to impair the completion of remyelination. Among them are factors related to a defect in OPCs activation and recruitment, or to

local inhibitors of remyelination. Theoretically remyelination can be blocked at any point in the remyelination process: oligodendrocyte survival, proliferation, migration, maturation, and/or myelin sheath formation. In MS lesions late in the course of disease, oligodendrocyte recruitment is deficient and appears to be the primary reason for poor remyelination in late stage MS. In late stage MS, remyelination appears limited by oligodendrocyte density, which could be a product of impaired survival, proliferation, and/or migration of oligodendrocytes. In lesions containing more oligodendrocytes, impaired oligodendrocyte maturation is a major problem for efficient remyelination of lesions. Beyond the oligodendrocyte recruitment and maturation, myelination also requires contact between axons and oligodendrocytes and creation of multiple wraps of oligodendrocyte processes around the axon, culminating in the myelin sheath. Another factor is that repeated demyelinating insults, as observed in the relapse-remitting form of MS, can exhaust the OPCs source so that remyelination failure may be regionally defined due to exhaustion of distinct progenitor pools (Podbielska et al 2013). While this study lists possible aspects of remyelination that could be affected, it is unknown whether it's a combination of these problems, or just one major mistake is occuring in MS. It seems however, that the main problem is less OPC differentiation into oligodendrocytes, which causes a lack of oligodendrocyte density. If the root cause of this problem is found, then a treatment method can be created to combat that and to keep the body's natural remyelination strong to combat the inflammatory effects of the disease. Research has been done on certain pathways that promote remyelination overall, such as Neurotrophins, Insulin Like growth factors, the Gp130 family of Neurotrophic Cytokines, the gene Interleukin-11, and Neuregulin 1 type III, (Zhang et al. 2011). There has also

been research done on certain inhibitors of myelination, such as Canonical Notch Signaling, the Canonical Wingless Pathway, and Bone Morphogenetic proteins (Zhang et al. 2011). Out of these growth factors, Neurotrophins show the greatest promise in terms of increasing functional recovery and remyelination. Neurotrophins (NTs) comprise a family of soluble mediators including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5. A study by Christelle Girard et al. found that "transplantation of fibroblasts expressing either BDNF or NT-3 has been shown to enhance axonal growth, OPC proliferation and myelination in adult rat spinal cords after injury. Moreover, transplantation of BDNF or NT-3-expressing Schwann cells into demyelinated mouse spinal cords leads to increased OPC proliferation and differentiation, remyelination and locomotor recovery. Interestingly, studies in the MS model experimental autoimmune encephalomyelitis (EAE) imply a functional role of BDNF in mediating axon protection in autoimmune demyelination. Remyelination and functional recovery have also been reported following transplantation of glial-restricted precursor cells (GRPs) expressing a multi-neurotrophin of BDNF and NT-3 into the CNS of rats subjected to spinal cord injury," (Girard et al. 2005). However, while these studies provide evidence that neurotrophins are effective at promoting regeneration of the injured spinal cord, the relative contributions to these outcomes of effects on neurons versus glia remain to be fully defined. I propose that that we combine the two studies, and research further the full effect of transplanting neurotrophic factors into MS mice models, for if we can use this to increase OPC differentiation, which seems to be the biggest factor preventing remyelination in MS, then we can treat MS without leaving the body susceptible to diseases.

Developments in Spinal Cord Injuries Treatments, Daniela Lastras, Sophomore

Spinal cord injuries (SCI) affect more than 250,000 people in the United States with thousands of new cases reported annually. Motorcycle accidents are currently the leading cause of SCI which includes any damage to any part of the spinal cord or any nerves (Darian-Smith, 2009). SCI can severely impair someone's life by reducing one's quality of life and productivity and by creating emotional and financial difficulties to patients. As of now, there is no cure to SCI, however there are a variety of treatments that can be used to alleviate some of the burdens that occur after SCI (Wang et al., 2016).

After a SCI occurs, the body will naturally do its best to heal the new wound. However, the healing process of SCI often leads to the loss of neurons. The acute phase of SCI healing occurs seconds, minutes and hours after the injury happens, this is also known as the primary mechanical trauma. Seconds after a SCI takes place, hemorrhage, ischemia and hypoxia will occur, this means that there will be an abundant amount of discharge of blood and there will be a lack of oxygen going into one's organs and tissues. In minutes, pro-inflammatory cytokines will reduce inflammation and glutamate cytotoxicity will send signals between nerve cells. The final step of the acute phase includes what happens hours after the injury, which is the release of free radicals which includes nitric oxide, this means that their will be an increase of blood flow. This final step of the acute phase also includes the release of the enzyme protease, which breaks down proteins. The subacute (chronic phase) of the SCI healing occurs days and weeks after the injury. In days, neuronal apoptosis (a form of programed cell death of neurons), astrogliosis (an increase in the number of astrocytes) and axonal demyelination (which eventually leads to axonal loss, through the loss of myelin which comes from oligodendrocytes on the central nervous system and Schwann Cells in the peripheral nervous system) will occur (Okano, 2010). An axon conducts electrical impulses away from the neuron's cell body. Astrocytes play an important role when it comes to guiding axons, supporting synapses and controlling the blood brain barrier and blood flow. Finally, in a matter of a couple of weeks severed axonal degeneration, cyst formation and permanent loss of spinal function will happen (Okano, 2010). The picture to the right demonstrates how glial cell formation looks like in response to an injury and inflammation. The glial scar barrier borders over regions of tissue damage and inflammation which is due to trauma, ischemia, cytotoxicity, infection and autoimmune inflammation. As one can see (Fig.1), astrocyte processes overlap at an extreme level, this creates a barrier that stops neurogenesis from occurring (Sofroniew, 2009). Due to the glial scar barrier, which protects and begins the healing process in the nervous system, the chosen method of promoting neurogenesis must happen before the permanent loss of spinal function.

One method to promote neurogenesis after SCI is to convert astrocytes into neurons through the elimination of the p53 and p21 pathways. Since there is an abundant amount of astrocyte cells near the site of the injury, converting these cells into neurons would be something ideal to do. Stimulation of p53 and p21 inhibits the cell cycle which results in no cell division, however the opposite is also true, elimination of p53 and p21 promotes the cell cycle which results in cell division (Wang et al., 2016). Therefore, elimination of p53 and p21 increases neurogenesis. The deletion of p53 promotes SOX2 dependant iANBs (induced adult neuroblasts), which is part of the process of converting astrocytes into neurons. SOX2 plays a critical role in the maintenance of embryonic and neural stem cells and it also increases number of DCX cells, which are involved in the movement of nerve cells. Neurotrophic factors such as GDNF, FGF2 and BDNF can be added to the iANBs at an immature stage

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Figure 1, from Sofroniew, 2009
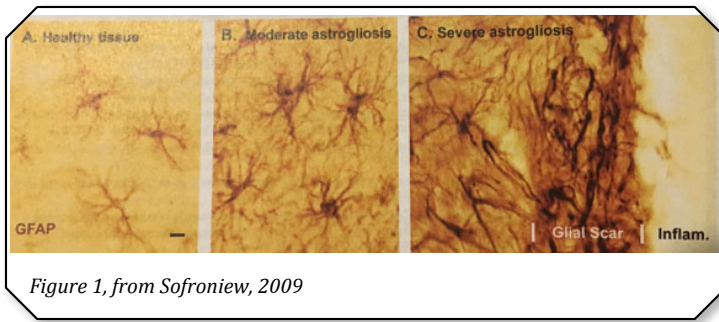
to convert new neurons (Figure 2). A lentivirus injection can deliver certain genes such as SOX2, GFAP-Cre, Bdnf, Nog and shRNA in the adult spinal cord, these genes mainly target astrocytes in the spinal cord to promote neurogenesis. One can also optimize procedures (such as injection method), to create the best environment possible to create diverse neuronal subtypes. In addition to optimizing procedures, valproic acid can also be used to promote neuronal maturation.

Another way to promote neurogenesis before the glial scar becomes predominant, is to transplant stem cells by using ES and iPS cells (Okano, 2010). Stem cells have the capability of reproducing themselves indefinitely. In certain conditions, stem cells can differentiate into specialized cells of one or more types and they can even regenerate damaged tissues. iPS cells are induced pluripotent cells, this type of cell can differentiate into many different cells by turning back the clock and reprogramming them to act like ES cells or otherwise known as an embryonic stem cells, which are capable of differentiating cells of any type. Scientists are able to turn back the clock
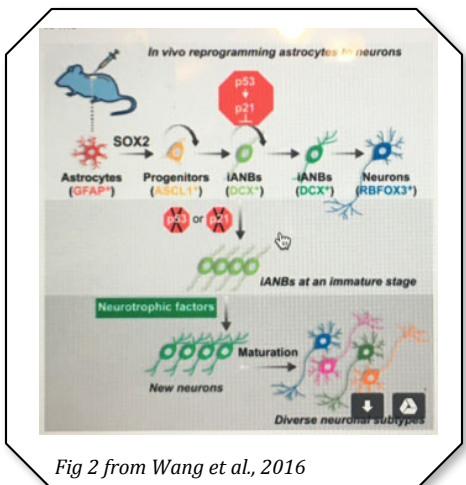
by converting the stem cell to a progenitor cell, then to a skin fibroblast cell and finally to an iPS cell. These iPS cells are then programmed to become neurons. To promote the increase of the transplanted neural stem cells (NSCs), neurospheres, a culture system composed of free-floating clusters of neural stem cells, from mouse ES cells can be expressed through certain genes. These neurospheres promote axonal growth, remyelination (creating new myelin sheaths in the central nervous system) and angiogenesis which results in locomotor functional recovery after SCI. Myelin sheaths play an important role in increasing the speed at which a nerve impulse can travel along an axon (Okano, 2016). Another way to promote neurogenesis is to transplant fibrin matrices containing NSCs and growth factors that can enhance graft survival, which is an estimate of the probability of a transplant functioning in SCI. The graft derived neurons are able to extend long distance axons and are able to restore disrupted neural circuits. Tissue engineering neural network constituted in vitro by co culture of gene modified Schwann cells (cells that produce the myelin sheath around neuronal exons) and NSCs in a gelatin sponge scaffold showed that the constructed neural network could integrate into the host functional neural network, and could promote axonal regeneration of host neurons (Dell'Anno et al., 2018).

A more modern way of promoting neurogenesis after SCI consists of using 3D printers. These 3-D printers can make implants that can be placed at the cite of the SCI. Unlike most printers, the printers that specialize in making these implants can print down to one micron, which is very precise when concerning these implants. The precision of the implants

allows scientists to print out the gray matter in the middle of the spinal cord and the protective white sheath of myelin nerve cells around the spinal cord. The printer is able to make small implants for rats by printing out softgels filled with neural stem cells. These implants can then be placed at the cite of the injury and regenerate the nerve cells and axons, which can then grow and connect to the healthy spinal cord (Niiler, 2019). These implants also allowed for some functional motor control of the rodents hind legs. Fully printing the central nervous system (CNS) has yet not happened because the CNS is too complex and technology has yet not reached that level of complexity. However, 3D biomimetic hydrogel scaffolds can fit the dimensions of a rodent and human spinal cord and offer a means of enhancing CNS regeneration. These hydrogel scaffolds contain neural progenitor cells (NPC), these cells support axon regeneration and synaptic connections (Koffler et al., 2019). The picture to the right shows a simple version of transplanting an alginate scaffold instead of a hydrogel scaffold into the SCI. The printer is capable of printing 2mm hydrogel implants in 1.6 seconds, so given the human MRIs of SCI, scientists were able to print out larger implants in only ten minutes. The short time in which it takes to actually print out the implants is essential, because the glial cell barrier that results in the permanent loss of neurons occurs in weeks (Boissonneault, 2019).

SCI can severely impair someone's life by reducing the quality and productivity of one's life and by creating emotional and financial difficulties to patients. Due to the glial scar barrier and the

Fig 2 from Wang et al., 2016

Fig 3
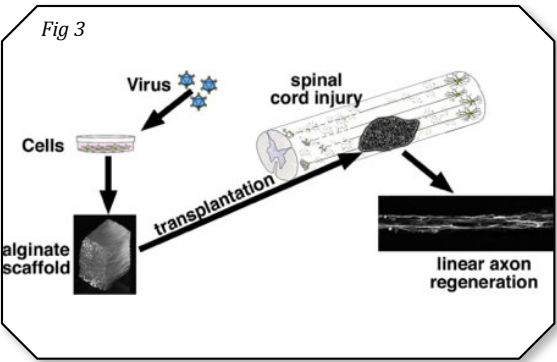
healing process of SCI, treatment must be done in a limited amount of time which. As of now, there is no cure to SCI, however there are a variety of treatments such as neural stem cell transplants and injections that can be used before the permanent loss of spinal function occurs to alleviate some of the burdens that occur after SCI (Wang et al., 2016). (Citations available upon request)

Effects of Triclosan, by Sarah Brizzi, Freshman

Fig. 3

Triclosan (Figure 1) has been used as an antibacterial chemical for several years, beginning in hospitals, to products all over the world that are used every day such as hand soap, Colgate toothpaste, even sprayed on toys for children. The FDA has recently banned and set various regulations for the use of Triclosan in many products, yet it is still found in various kinds of toothpaste. Some companies have removed Triclosan from their products with the suspicious views upon it, while some best-selling brands still incorporate the chemical into their product. People are blinded by brands who claim that their product kills the most germs. According to Martin, a survey by the Centers for Disease Control and Prevention found triclosan in over 75 percent of Americans' urine and believe it may lead to antibiotic resistance, causing researchers to question if Triclosan exposes a greater danger to consumers than they know. Claimed to have more risks than benefits, we wonder, is it efficient without causing harm to the user (Martin, 2011)? With articles being read by a wide population, are people ignoring the harm they put themselves in when using products with triclosan? With the lack of successful and efficient

studies determining the association between triclosan and child adiposity, Geetika Kalloo at Brown University conducted a study attempting to discover if triclosan affected the endocrine system and gut microbiome to influence obesity. Kalloo believed if triclosan impacted the endocrine system and gut microbiome to influence adiposity, children exposed to higher concentrations of triclosan would have a lesser amount of triclosan in their urine due to the fat cells storing the triclosan within the body, which implies increased adiposity (Kalloo et al., 2018). The gut microbiome contains several microorganisms and various types of bacteria helping the digestion of specific foods, the production of certain vitamins such as vitamins B and K, and plays a large role in the immune system protecting the body from other foreign microorganisms (European Society of Neurogastroenterology & Motility, 2016). The mothers studied were all 18 years and above, recruited from the Cincinnati area, fulfilling the many requirements. Examination of the urinary triclosan concentration occurred twice during pregnancy, annually from ages one to five and finally at year 8. With 468 eligible participants, 389 of the women were carrying only one child. Urine samples were collected in polypropylene cups, and stored at -20 degrees Celsius prior to analysis. Using body-mass index converted according to sex, waist circumference and bioelectric impedance revealing the body fat percentage child adiposity was evaluated at 8 years. By determining the mean and median urinary triclosan concentrations for each analysis and body-mass index, Kalloo then calculated the correlation coefficients and associations between child adiposity and each urinary sample concentration results. Researchers

viewed participant's medical history as well as questioned the participants through an online examination to determine factors that could alter the results. Sociodemographic variables such as wealth, marital status, race, and education were collected besides perinatal factors such as feeding methods, a way of birth, genetics, vitamin use and more. A weak association was found between the association of child adiposity and urinary triclosan concentration. Also, the results did not show a large difference according to the sex of the child. For girls, the largest correlations were found in prenatal triclosan concentrations and for boys, it became difficult to determine due to the fluctuating results, though adiposity does vary greatly between males and females (Kalloo et al., 2018). Participants were gathered from the Cincinnati area possibly affecting the accuracy and diversity of the results due to the environment.

Triclosan' main purpose has been to kill pathogenic bacteria that can cause disease, yet recently triclosan resistance has been recognized in several bacterial species, and little has been studied on the resistance determinants in the pathogenic bacteria. The researchers sought to assess the distribution of triclosan-resistant determinants and analyze the enrichment of likely pathogenic genera in areas and environments polluted with triclosan. To determine the distribution of genes that verify triclosan resistance, Khan utilized computer modeling, in silico genome analysis of the 183 FabI bearing the most common human pathogens and

Fig 1 Triclosan
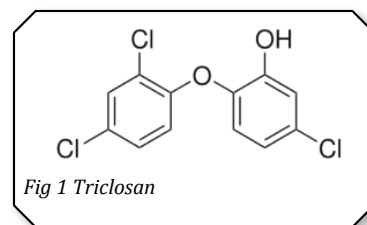
Mentor:
Anthony Hay, Ph.D.

Associate Professor
Department of Microbiology
Cornell University

soil-borne plant pathogenic bacteria for his study. Bacterial strains were grown and exposed to certain antibiotics allowing Khan to evaluate which strains obtained antibiotic resistance caused by triclosan exposure. In order to determine if triclosan resistance could be assessed from present triclosan-resistant genes, Khan evaluated 17 laboratory strains, conducting a search to determine the similarities and associations between individual human-associated pathogenic bacteria or soil-borne plant pathogen genomes and the triclosan-resistant gene reference database which was created to predict triclosan resistance in pathogenic bacteria. Candidate triclosan-resistant bacterial genes were identified and cloned in E Coli. E Coli cultures were then assessed for triclosan resistance. In order to evaluate triclosan-resistant determinants, in silico analysis referring to computer-based evaluation was conducted on selected human pathogenic bacterial strains and soil-borne plant pathogenic bacteria. Soil samples contaminated from triclosan were tested to seek the abundance of triclosan-resistant pathogens. The genome-wide analysis displayed that the predicted triclosan-resistant genes were abundant in the majority of human-associated pathogens and soil-borne plant pathogenic bacteria. The

microbiome evaluation showed that pathogenic genera that obtained triclosan-resistant determinants are present in the environments polluted with triclosan. The triclosan resistant determinants are also associated with the antibiotic resistant strains of bacteria. Through these discoveries, it can be concluded that triclosan is not as useful as once believed to be, because of dangers that go beyond triclosan resistance such as antibiotic resistance from triclosan exposure. (Khan, Roy, Choi, & Lee, 2018).

In an earlier study, Khan conducted similar research analyzing environments to find an abundance of resistomes and triclosan exposure. Khan aimed to inspect the triclosan resistome from the soil metagenome, analyze other antibiotic resistance due to triclosan, conduct profiling of prototypic and triclosan-resistant metagenomic ENR diversity from several areas, and examine particular ENR abundance in environments heavily exposed to triclosan. The methodology incorporated includes determining the minimum inhibitory concentration, DNA manipulations, metagenomic library construction and evaluating triclosan-resistant clones, subcloning of triclosan-resistant clones, phylogenetic analysis and comparative searches for an abundance of resistomes. From the research, it was determined the environments tested were highly polluted from triclosan. Khan's studies exemplified that metagenome-derived diverse ENR variants correlating to triclosan resistance are prolific in natural soils and pathogenic microorganisms. The large quantity of triclosan use and exposure to microorganisms leads to alterations in ENr and the target enzyme. The excessive triclosan concentrations found, along with the results that uncontrolled use of triclosan will lead to an enrichment of triclosan and antibiotic-resistant bacterial pathogens in the environment causes a great stress.

Gao predicted if Mice are exposed to a high dose of triclosan for thirteen

weeks, then their gut microbiota and microbiome will be extremely perturbed because of a drastic change in bacterial families and new resistomes. Gao aimed to determine if triclosan could be a threat to the gut microbiome of species, and study the alteration of the gut microbiome due to triclosan exposure. The mice were randomly split into an experimental group and a control group. While both groups were given normal pellet food, the control group was given fresh water, while the experimental group was given water with a high dose of triclosan for thirteen weeks. 16S rRNA sequencing was utilized to test both groups' feces, allowing the researchers to examine the bacterial families left and altered. Shotgun Metagenomics was used to compare and analyze genes of the bacteria in order to find antibiotic resistance. Results show a weaker microbiome because of less diversity of bacterial families and an increase in metal and antibiotic resistomes. Triclosan also changed the composition of the microbiome. Thirteen-week exposure greatly transformed the microbial community, shown by drastic shifts of bacterial families. The bacteria became more resistant to stress and any changes in its environment. Figure 1 shows the implications of triclosan involved with killing bacteria and promoting the growth of others, changing the biodiversity. Some bacterial families died off, while others were reinforced, those that were reinforced most likely imply triclosan and antibiotic resistance. This study exposes the mice to a strong dose of triclosan for 13 weeks, rather than humans who are exposed to triclosan over a longer time period and more gradually, yet does not invalidate the study at all. The growth of certain bacteria, along with the decrease of others depicts how greatly triclosan acts to remove bacterial diversity and reinforce antibiotic resistance in the surviving bacteria (Gao et al., 2017)

(Citations available upon request)

Discovering the Truth Behind Cerebral Palsy, by Sarah Mandelkow, Sophomore

Overview of Cerebral Palsy:

When people hear the words cerebral palsy they think of a brain injury, but recent studies have proven this disorder to be more complex. Cerebral palsy affects "2.0-3.5 per 1,000 live births" and this condition affects basic motor-functions, due to a loss of connection to the part of the brain that controls movement (Zarrei et al., 2018). Normal functions are delayed in people who are affected by cerebral palsy, but each person experiences a different level of involvement. This disorder is most likely to occur around the time of birth since the developing brain is in its most vulnerable state. The areas in the brain that have to do with movement are the cerebellum, the motor cortex, and the basal ganglia, if any of these areas become damaged the result could be cerebral palsy. (Fig. 1) There is a possibility that some people are more susceptible to damage in these specific areas, since their could be a disruption in this person's genes. The cerebral cortex is responsible for the movement of the skeletal muscles, and these muscles help perform basic functions. For someone who has cerebral palsy simple day to day, tasks are difficult to perform because they lack control over their skeletal muscles. However, cerebral palsy is not a disorder that gets worse over time, instead the challenges people face when they have this condition stay not having any control over the way the muscles move. Because the brain's communication is disconnected from the muscles, they can become either hypertonic, having increased muscles tone, or hypotonic, having decreased muscle tone. Respectively these muscles react with greater or lesser efficiency causing people to have less control over their voluntary muscle functioning. The cerebellum is also affected when the brain undergoes this type of injury. It is this part of the brain that controls balance and makes your muscles work together. Therefore, when the cerebellum is affected someone can have trouble keeping their balance and the movements of their muscles may not be organized to carry out the motor planning necessary to achieve a specific task. As tonal changes extend throughout a person's life, muscle integrity becomes impacted. Hypertonic muscles can experience shortening resulting in contractures, hypotonic muscles may eventually not respond to nerve impulses. A decrease in Brain Derived Neurotrophic Factoring, or BDNF could also be cause of these muscles not receiving nerve impulses. Since BDNF is responsible for the maintenance of neurons a lack of this could result in neuronal death (Baydyuk et al, 2014).

Categories of the Disability:

Cerebral palsy is just one general term that is used to categorize people with physical disabilities, but cerebral palsy refers to much more than just one kind of disability. There are multiple levels to this condition, some who has cerebral palsy are highly affected while others could be very minimally affected. There is a system, called the Gross Motor Function Classification System that is used to determine the there, are other subdivisions of cerebral palsy which are diagnosed based on how many muscles are impacted. Monoplegia is the least severe type of cerebral palsy, as it only affects one extremity. Then there is diplegia, which impacts bilateral extremities. Another classification of cerebral palsy is hemiplegia, where only half of the body is affected. In one study "this CP subtype accounted for 37.4% of CP cases in a Canadian cohort," showing this one of the more common types of cerebral palsy (Zarrei et al., 2018). The most severe case of cerebral palsy is quadriplegia where all extremities are affected, most often muscles of the trunk, face, and mouth are also impacted. Each one of these categories is further defined by the quality of muscle movement. Spastic cerebral palsy involves hypertonic muscles which are tight, because of the damage to the cerebral cortex. The messages that are being sent to the muscles are incorrect since the brain is damaged and then the muscles tighten and resist voluntary movement. This results in typically upper and lower extremity posturing, commonly seen as flexion of all major joints. Non Spastic cerebral palsy involves muscles with fluctuating tone. Many classifications exist due to the complexity of the brain and the extent of damage to any given area.

Predisposing Factors:

Studies have shown that there are some predisposing factors that are related to cerebral palsy that occur before pregnancy, during pregnancy, labor, immediately after birth or a few months

Fig1

Stiff muscles (spasticity), associated with damage to or developmental differences in the **cerebral cortex**

Uncontrollable movements (dyskinesia), associated with damage to the **basal ganglia**

Poor balance and coordination (ataxia), associated with damage to the **cerebellum**

Mixed, a combination of two or more types, associated with damage to **multiple areas of the brain**
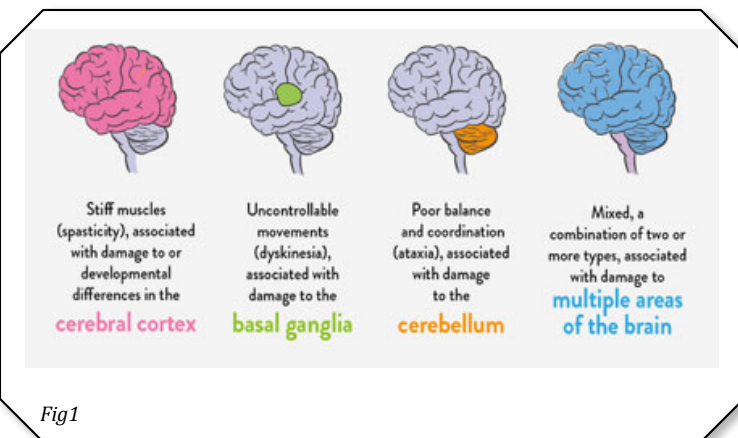
after birth. A risk that can occur ahead of pregnancy involves a problem relating to the menstruation cycle, the regulation of the cycle could have been thrown off. Another factor in this stage has had to so with there being too large or too small a period of time in between pregnancies. Then there was a study that showed people living in a lower social status were more at risk than people who lived in a higher social class (Dolk et al, 2001, Dowding and Barry, 1990). Risks related to the prenatal stage involve the carrier of the child being diagnosed with pre-eclampsia, this condition can cause premature birth and with this comes the chance of infection. In one group of data that was made up of genes that were affected by cerebral palsy, "49.7% of cases were pre-term deliveries" (Eyk et al, 2018). If there are more than one pregnancies at once or a twin pregnancy where one of the twins does not survive then cerebral palsy becomes a potential risk. During labor, there is the possibility of the umbilical cord getting wrapped around the baby's neck and then asphyxia could occur. Asphyxia can also take place if "cephalopelvic disproportion" occurs during the birthing process (Reddihough and Collins, 2003). Towards the end of pregnancy or at the time of birth, there is a chance of infection taking place and this could negatively impact the brain. The less the baby weighs at birth, the more of a risk the child is at for being diagnosed with cerebral palsy. If the baby did not undergo the proper amount of time needed to grow inside the womb then the child is more at risk for developing cerebral palsy. Once the baby is born there is a risk of the child

undergoing a seizure or developing a "respiratory disease" which would, in turn, increase the possibility of being diagnosed with cerebral palsy (Reddihough and Collins, 2003).

Possible Causes:

The most well known possible causes of cerebral palsy can arise at any stage of a person's life, and they have to do with brain damage, complications at birth, and "congenital brain malformations," but there's also a possible genetic connection to this condition (Reddihough and Collins, 2003). In the prenatal stage, a possible cause could be that there was an abnormal formation of the brain. Due to "brain imaging" scientists have found an artery could have been blocked before birth, thus causing cerebral palsy (Reddihough and Collins, 2003). During the delivery process, labor could be interfered with in some way or the umbilical cord could be shifted in a way that results in hypoxia. "Neonatal encephalopathy in infants of ≥34 weeks gestation" causes cerebral palsy because the baby can't breathe properly, their reactions are slow, and they are prone to seizures (Reddihough and Collins, 2003). The time after birth is when the baby can get an infection, which can cause cerebral palsy. This is also the time when newborns can
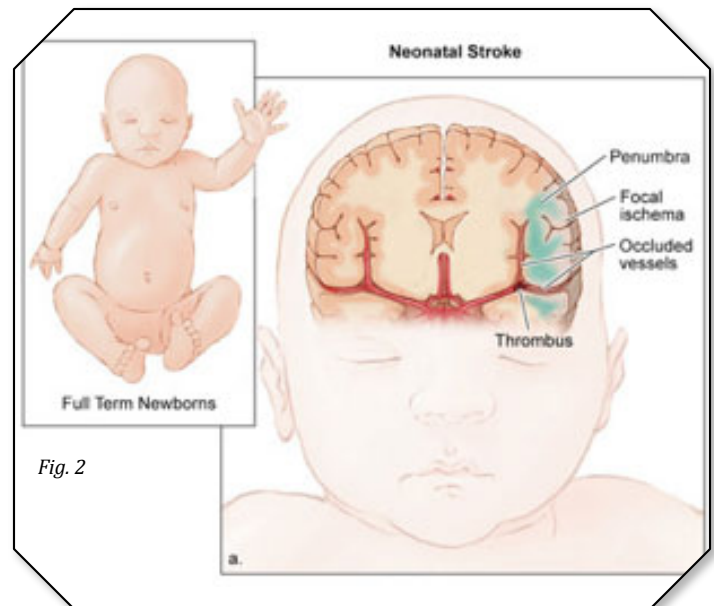
Fig. 2

develop low blood sugar or jaundice, both of which make the child more susceptible to developing cerebral palsy. After the neonatal period, infection can become another cause, as well as a stroke (Fig. 2), or trauma that occurs unexpectedly or results in a near death experience. In underdeveloped countries, malaria, meningitis, and other diseases remain a risk, which is known to be a cause of cerebral palsy. One study found that there is a possibility of "gene networks and pathways contributing to CP" (Eyk et al, 2018). Meaning, cerebral palsy could be caused by the presence of a certain gene, which could be inherited from generation to generation.

Treatment Options:

In terms of treatments for cerebral palsy, there are a few preventative measures that can be taken, such as participating in physical or occupational therapy, but there is not yet a cure. The overall goal of the occupational and physical therapists are to manage muscle tone to obtain the greatest motor planning to complete functional activities. Each branch of therapy works on a different set of muscles and uses different tactics to achieve their goal. Before not much was known about the impact of physical therapy, but now we have discovered that muscle strengthening and "resistance training" helps to rebuild pathways in the cerebrum (Damiano, 2009). Physical therapists try not to
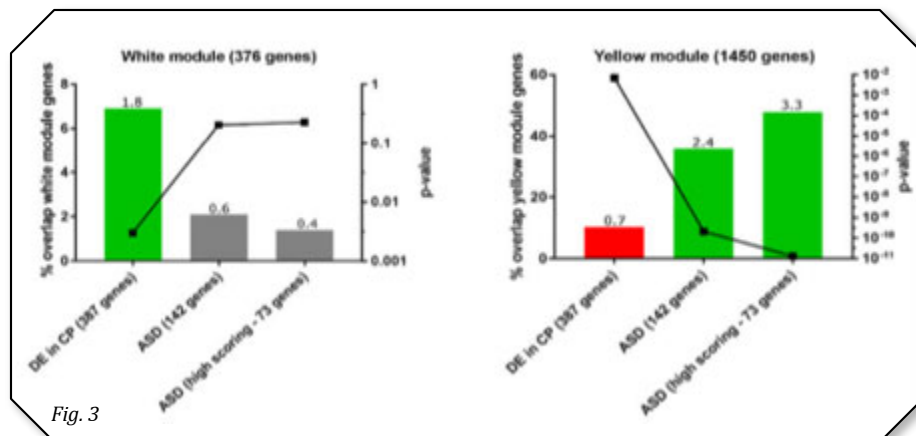
Fig. 3

overwork the muscles, but they want to get them moving in order to strengthen them. In addition to making the muscle stronger, physical therapists want to develop stamina and make the muscles work together to help with common tasks, such as ambulation. Results have proven that "the use of treadmills" in physical therapy can address both of these areas and help patients with cerebral palsy (Damiano, 2006). Physical therapists and occupational therapists work together on positioning to minimize contractures from the spastic muscles. Occupational therapists focus more on hand functioning, they can make splints, or teach skills that address activities of daily living, including feeding, dressing, bathing themselves, and work hardening. Some adaptations have to be made or compensatory strategies have to be developed in order for patients with cerebral palsy to find alternate ways to complete tasks. For example, switches could be added to a wheelchair for easier control over it, or a stylus can be made to access a computer keyboard. Wheelchair fitting for those who are non-ambulatory is worked on by physical therapists and occupational therapists. Speech therapists work on feeding, jaw control, or language production because cerebral palsy can result in tight facial muscles. Psychologists also work with people who suffer from cerebral palsy because they provide counseling for social-emotional support. Medications and some surgeries are also available to help control the symptoms associated with this disability.

Connection to other disorders:
Research has shown that there could be a possible connection between cerebral palsy, autism, and intellectual disability because the factors involved with cerebral palsy overlap with aspects related to these other conditions. One study that was conducted focused on multiple genes that were affected by cerebral palsy, by breaking them down into a white and yellow module. The group of genes that were in the white group was "enriched for markers of BDNF signalling" (Eyk et al, 2018). BDNF signaling is involved with the development of neurons and this signaling is seen when the brain does not develop properly because the signals are used to compensate for the brain's loss of function. The genes in the yellow group are "enriched for genes involved in developmental processes" (Eyk et al, 2018). The study found that both the white and yellow modules showed downregulated genes that shared a link with the "autism candidate genes" they were compared to (Eyk et al, 2018). (Fig. 3). These same genes were also compared to other disorders using the Nijmegen genome diagnostics for intellectual disability (ID), the database for autism and schizophrenia, and to the Simons Foundation Autism Research Initiative. The results showed the genes affected by cerebral palsy are associated with each disorder in some way, but the greatest connection is between these genes and the ones that are expressed in autism. The lowest connection is seen between the genes influenced by cerebral palsy and the genes affected by intellectual disability. (Fig. 4) The discovery of cerebral palsy sharing a link with other disorders creates a new theory and opens up the scientific world to discover new information on these disorders and maybe even lead to improving treatment options.
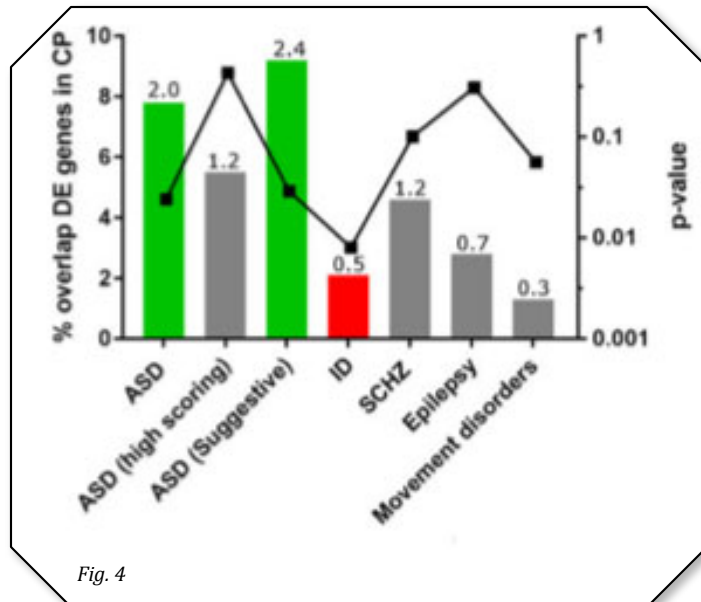
(Citations provided upon request)

Fig. 4

Brain Blood-Brain-Barrier Therapy in Preventing Amyloid beta Build-up and Production, Michael Mahoney, Sophomore

One of the most common and most damaging neurodegenerative diseases is Alzheimer's disease. Commonly referred to as AD, Alzheimer's causes tangles of plaque to build up in the brain. This plaque is toxic to neurons so the further the disease progresses the more damage it does. AD's toxic impact on neurons causes dementia which leads to a loss of memory, an inability to clearly think, and a loss of reasoning. Dementia caused by alzheimer's currently has no cure. Scientists trying to cure AD are looking at almost all parts of the brain. The hippocampus, where memories are made is a particularly high area of study. To understand AD brains scientist use cells in the hippocampi and test them for the changes as they develop Alzheimer's. As the cells develop AD, many changes occur within the cell. These changes range from large scale leaks in the membrane of cells in the sydodendric compartment, the cells in the interior of the neurons, (Bahareh, 2018) to a change in the way the metallic ions in cells end up contributing to the mass build-up of amyloid beta plaques (James Everett et al., 2018).

For the causes of AD, mainly non brain

related proteins and iron ions, to even get into the brain they need to pass through the blood brain barrier. Like each individual cell, the brain has a protective barrier protecting it from access to the rest of the body. This barrier is called the blood brain barrier and it stops many of the body's proteins from affecting the brain, specifically those carried in the bloodstream. If the BBB was to fail, anything carried in the blood would have access to the brain. In a study of how a gum disease, Periodontitis, can cause symptoms of Alzheimer's, researchers found that the pathogens from the disease ended up exposed in the brain. While the researchers did not specifically look for an opening, they concluded that the pathogens had broken through the Brain blood barrier or BBB (Vladimir Ilievski et al., 2018). This leads to the question whether that all cases of AD causes a break in the BBB and in the membranes in the deterioration in the sydodendric compartment of neurons.

When the BBB is broken effects can be numerous. The BBB is needed to maintain the chemical balances in the brain, and when it is broken the consequences include: a reduction in the flow of cerebral blood and damaged or slowed haemodynamic responses (M. D. Sweeney). Without the BBB toxic molecules from the blood, cells, and microbial agents would end up circulating freely in the brain. To find if the BBB was broken/damaged in post mortem tests of AD, researchers used capillary leakages as a marker as these are a very common feature in Alzheimer's development. The researchers also used degeneration of cells that were associated with the BBB, circulation of leukocytes and red blood cells, and aberrant angiogenesis and other changes in molecules. In the post mortem samples taken all showed signs of BBB breakage. This was indicated by accumulation of fibrinogen, thrombin, albumin, IgG, and hemosiderin (these all being blood cell-derived proteins) in the prefrontal cortex and in the hippocampus. All of the previously listed blood derived proteins were also iron containing proteins (M. D. Sweeney). The presence of iron in the core of Alzheimer's amyloid β plaques was shown in a study of X-ray breakdown of the plaques (James Everett et al., 2018). With iron compounds flowing through the damaged BBB in Alzheimer's and iron being an important element at the core of the amyloid β plaques naturally blocking the excess iron input would the next natural step to at least slow the production of amyloid β and the tangles that it creates.

A study on AD therapy through the blood brain barrier was last thoroughly investigated in 2018. In this study it was noted that IgG, ferritin, and dextran needed active transport across the BBB to enter that brain, but at the time the study of blood brain barrier breakdown in Alzheimer's cited previously was not yet published. They also noted at the time there was no proof of "massive disruption of BBB in patients with AD" (Marques et al., 2017). After noting the lack of proof of massive disruption in the BBB, researches stated that activated protein C could help protect the brain but did not test this theory (Marques et al., 2017). Researchers detailed activated protein C could help maintain the integrity of the BBB along with lessening the neuroinflammation, and decreasing the apoptosis of endothelial cells in the brain (Marques et al., 2017). Activated protein C in Alzheimer's as a BBB protective agent seems to be largely unexplored.

While the research on activated protein C used to stabilize the BBB is limited, the microglia, which are the equivalent of the the brains autoimmune cells, should be protecting and repairing the BBB when it is damaged. Scientists studying the brain in the event of a injured blood brain barrier stated that the microglia quickly repaired small gaps in the BBB and explained their presence to be central in the repair and maintenance of the BBB (Nanhong Lou et al., 2016). However in AD brains, the microglia do not serve this purpose. While in a non AD brain the microglia repaired the BBB in minutes after damage, they could not repair the damage in AD. Alois Alzheimer, the namesake the disease, noted that the microglia had developed many fibers (Alzheimer et al., 1995). Along with the physical appearance and composition of the microglia changing the role of the microglia in the brain drastically (David V Hansen et al., 2017). In AD brain, the microglia undergoes microgliosis, a process in which the microglia accumulate in reaction to an injury (Sauders, 2007). However instead of helping repair the tissue in the CNS the microglia often will cause more harm than good. When the microglia are activated in a large concentration in a small area, they can cause neural inflammation in the brain. The microglia are also linked to causing the "engulfment of neural synapsis" (David V. Hansen et al., 2017). In a 2017 study on the microglia in AD, researchers described how, when amyloid β is beginning to accumulate, some of the microglial processes to help repair damage in the CNS becomes static. The researchers also found that the older the plaques were, the less the microglia seemed to function. The study detailed that with plaques over days or weeks old the microglia surrounding them would no longer congregate in groupings or spread out around the plaques. Most importantly the microglia show no traits associated with the morphological

adaptation that occurs when healthy microglia detect the amyloid β plaques or tangles. The microglia show an increase in apoptosis, which is programmed cell death that leads to a mass depletion of cells. As large numbers of microglia that were in the area of the plaques become static and die other microglia surround the plaques to try to break them apart. The microglia that remain healthy attempt to compact the plaques which could make the plaques less toxic. This also prevents the addition of more amyloid β from joining the long string that has already been surrounded. The effectiveness of the microglia seems to be affected by age as the effective way of compacting the amyloids was observed much more often in early onset patience of Alzheimer's then in their older counterparts (Hansen et al., 2017).

This aged based assumption proved correct as, in a 2013 study of age in relation to the microglia, it was noted that the microglia in the elderly were more likely to become "primed". This means that they were more likely to inflame the area that they were trying to repair. This occurs because the older you are the longer the activation of the microglia lasts despite the importance or severity of the situation that they were

activated for. A small task could activate a microglia for hours or days despite the damage being fixed in minutes. When the microglia is active, but has no problem, is the time when the neural inflammation begins. When a large amount of amyloid β is being produced at a constant rate, a large amount of microgels are activated. As stated before, the microglia are not able to break apart the strands of plaque so they are active and doing nothing which contributes to the severity of the neural inflammation that is a symptom in AD especially in the older patient (Diana M. Norden et al., 2013).

Despite the implied useful effects that limiting the activity of the microglia would bring in Alzheimer's, there is very limited research. However quite the opposite is true in multiple sclerosis, also known as MS, has a large amount of research pertaining to neuroinflammatory reduction based on limiting the microglial activities. In a 2013 study of the effects of dipyridamole on MS patients, it was noted that the researchers could not find any studies published where the effects of dipyridamole were covered, and, to the extent of my knowledge there has been little to no research on reduction of microglia activation in late onset AD

patients or even on lab rats despite the large amount that is present for MS (Sloka et al., 2017).

The complexity of the Alzheimer's disease has spread the research on this topic thin. Many scientists believe that the answers to ending the neurodegenerative disease lie different parts of the brain. If given the resources to investigate AD, possibly the most rewarding area to investigate would be the BBB and how to stop the proteins that cause AD from even entering the brain. Stopping the flow of ferritin from entering the brain through methods of activated protein C additives or microglia targeted drugs or treatments would help prevent AD from manifesting and at least, if nothing else, the strengthening of the BBB would at least delay the disease. The relatively recent idea of the importance of the BBB in alzheimer's means that the idea is not nearly full investigated, and using modern research developed for MS and other neurodegenerative/inflammatory diseases could lead to new knowledge on how to delay or delay prevent Alzheimer's disease from developing in the brain.

(Citations available upon request)

Human Behaviors to Halt Climate Change, Katelyn Hartigan, Sophomore

Energy Use Crisis and Solutions
As the state of the environment is deteriorating, researchers are looking for different ways to help halt climate change. Humans are most to blame for the many environmental issues that the modern world is facing. One technique that researchers are becoming more interested in to help people view climate change with a more pressing and urgent attitude is psychology (Attari et al., 2016). Psychology can improve the way people use energy in their daily lives. If people view their personal energy and resource use more accurately, they will have a better idea of what they can do to reduce their energy use.
Threats in Freshwater Ecosystems and Possible Solutions

Human activities lead to many different threats to the environment, specifically freshwater ecosystems and watersheds. Pollution in these ecosystems and watersheds cause more problems the longer we allow them to be polluted. In each polluted water system, there are multiple threats which interact with each other, making up more complex problems. Many different human actions cause these environmental issues, including resource extraction, habitat alteration and fragmentation, contaminants, and non-native species. Different professionals specialize in different threats to the ecosystem, however, there are very few people who are researching the way these different threats impact each other, and how the

Table 1

Categories	Study 1 (N = 717)		Study 2 (N = 685)	
	Self	Americans	Self	Americans
Turn off lights	19.5	13.0	13.6	10.2
Drive less	19.3	31.8	19.3	31.8
Turn off appliances	10.9	7.8	12.6	10.7
Change setting on the thermostat	9.1	4.6	10.7	5.7
Sleep/relax more	7.3	4.6	1.8	1.3
Use appliances less	5.4	4.6	8.3	4.7
Unplug appliances	5.0	2.8	7.0	4.5
Conserve water/energy	4.6	4.5	4.2	1.5
Use energy efficient bulbs	2.8	3.6	2.8	3.6
Consume less	2.7	4.0	0.9	2.2
Other (each only mentioned once)	2.4	1.8	4.5	3.2
Use efficient cars/hybrids	2.2	2.2	2.3	6.7
Use efficient appliances	1.8	2.9	3.9	3.1
Change my lifestyle	1.8	2.5	1.3	0.9
Buy green energy	1.3	3.2	1.6	3.4
Buy green products	1.1	1.0	0.3	0.0
Eat green	1.0	1.0	0.6	0.3
Recycle	0.7	1.4	0.9	1.5
Insulate my home/weatherize	0.4	0.4	1.3	1.5
There is no way/I don't know	0.4	0.4	0.1	0.0
Awareness/education; more attention	0.1	1.4	1.8	2.8
Phase out inefficient technologies	0.1	0.4	0.0	0.6

course of action taken to treat these them may need change based on the way they interact (Craig et al., 2017). If more research went into understanding the interactions of environmental issues, the findings of the studies could be used as a motivator to the public to illustrate how widespread environmental issues are.

Currently, most management of environmental issues currently focus on a single solution to a single threat, rather than the specific challenges that come along with paired or multiple threats. Scientists are often overwhelmed by the thought of handling multiple environmental problems at one time. It is suggested that if researchers and managers that specialize in dealing with different environmental issues work together and use their specialized knowledge to develop plans that are able to solve more than one issue at a time (Craig et al., 2017). By being exposed to new and differing opinions, researchers may be able to reduce, if not eliminate, any bias they develop on a particular issue or set of issues relating to the environment. By eliminating some bias

that develops during research, a more neutral depiction of climate change can be presented to the public.

The Population's Perception of Energy Use and Conservation

People often misjudge the amount of energy and resources they are using in their everyday lives. According to Worldwatch Institute, Americans make up less than 5% of the global population, however, use more than 30% of the

world's resources. If the rate at which humans are emitting greenhouse gasses is not reduced in the near future, the planet will be in crisis. When greenhouse gasses reach excessive amounts, the Earth will begin rapidly heating up which will have a wide array of consequences. However, using a variety of different techniques, the population can be educated as to how to consume less energy and emit fewer greenhouse gasses. Scientists and researchers that focus on climate change may also benefit from expanding the different array actions they take to reduce waste and save resources. When focusing on only one specific topic in climate change, scientists and researchers may lose track of the other actions they should take to help the environment. According to a study conducted by Shahzeen Attari in 2016, people perceived the techniques that appeared to be less applicable in saving energy to be more effective than techniques that appeared to be more applicable (Attari et al., 2016). People will prefer to adopt energy conservation efforts that they perceive to be more applicable to their daily lives than the conservation behaviors that are seemingly less applicable, or appropriate, to their daily lives. By changing the perceived applicability of different energy saving behaviors, people may be more motivated to incorporate these behaviors into their

Fig 1
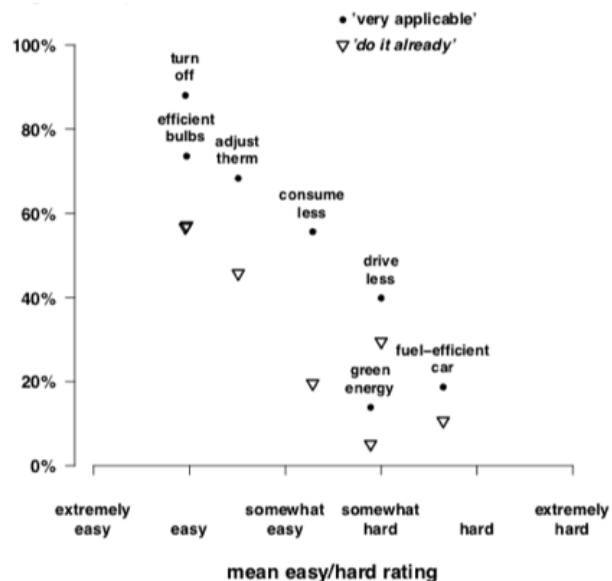
daily lives. In the survey, it was also noted which energy conserving actions people recommended for themselves, and which they recommended for other people. People in the survey concluded that they should adopt actions that were seemingly more applicable to everyday life, such as turning of the lights, and also answered that Americans as a broad population should adopt less applicable actions such as driving less, (refer to Table 1). This supports the conclusion that the participants of the survey suggest other Americans should take more drastic and effective measures to reduce energy consumption and greenhouse gas emissions, however, they don't want to be the ones making these changes. If people can change their perception as to how they think of their personal energy consumption, there would be noticeable results in the efforts to reduce the consumption of energy and production of fossil fuels. Shahzeen Attari conducted another study in 2016 in which participants of a survey were asked to rate the applicability of seven different actions to everyday life, and then were asked if they already have adapted the specific behavior. It was found that even when an action was thought to be very applicable, only around 50% of people already had adapted the action (refer to figure 1). As demonstrated throughout the paper, people have an inaccurate view of their energy usage, and in order to fix the public's perception of energy consumption and the production of greenhouse gasses, people need to be taught more about different behaviors that can reduce energy usage, and different activities that use large amounts of energy (Attari et al,. 2016).

The Public's Perception of Energy Usage and Conservation

Humans have been producing CO_2 at a rapid rate, and if the carbon emissions are not reduced, the quality of life for humans will be affected. In a national survey conducted by Shahzeen Attari in 2010, 505 participants were asked to report their perceptions of energy usage for a variety of different daily activities. The participants were asked to evaluate

the amount of energy these specific tasks required. The results showed that people would slightly overestimate the amount of energy that low energy activities utilized, and greatly underestimate the amount of energy that high energy activities required (Attari et al.,

Fig 2

2010). The participants of the survey also were asked about the amount of energy they thought a certain behavior could conserve, and then compared these results to the amount of energy that actually is saved. As shown in figure 2, people had a more accurate perception for the energy that can be saved by taking simple yet frequent actions, whereas they were less accurate in estimating how much energy could be saved by making larger efforts less frequently. The research who conducted the study concluded that if people had a more accurate perception of their energy use and saving, there would be more potential for a decrease in CO_2 emissions caused by daily activities. In the survey it was also found that those with stronger pro-environmental feelings had a more accurate view of their energy consumption and savings than those who did not express these feelings. This is most likely because they are more aware of different energy utilizing activities. These results demonstrate that when people have more knowledge about the energy they are using, they are more likely to choose energy saving techniques that prove to be effective (Attari et al,. 2010). The main goal of this study was to prove that the public currently has a warped perception
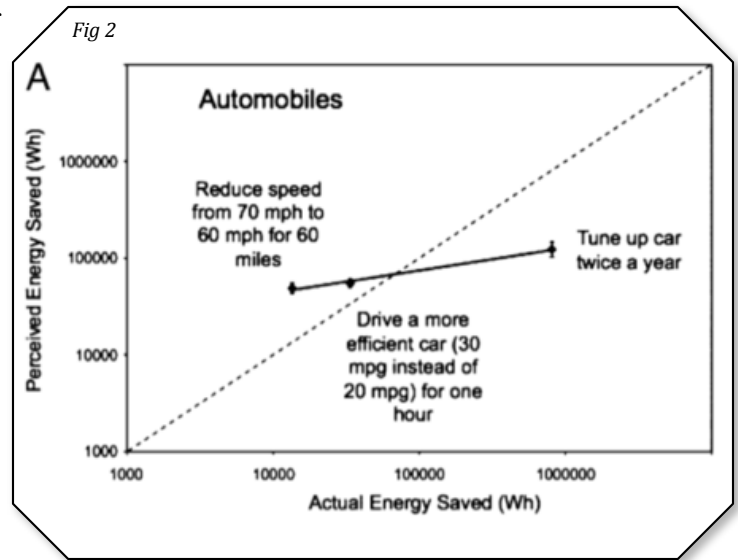
of energy consumption and savings, and with more education and awareness, a better perception of energy usage could reduce CO_2 emissions.

Problem with the Solutions?

In order to help halt global warming caused by CO_2 emissions, people must change the way they use energy. It is necessary that the population is smarter about the way they're using energy, and the way they think of global warming -- less of a political opinion and more as an issue that will affect everyone if not resolved. People have very different opinions about climate change and what measure should be taken to prevent its further progression. Some people have yet to accept the reality of climate change, while other accept it yet refuse to take any action to stop it, and there are also those who are doing all they personally can do be environmentally friendly. The scientific community has already accepted that humans are the main force behind climate change, climate change will cause atmospheric

Fig. 4

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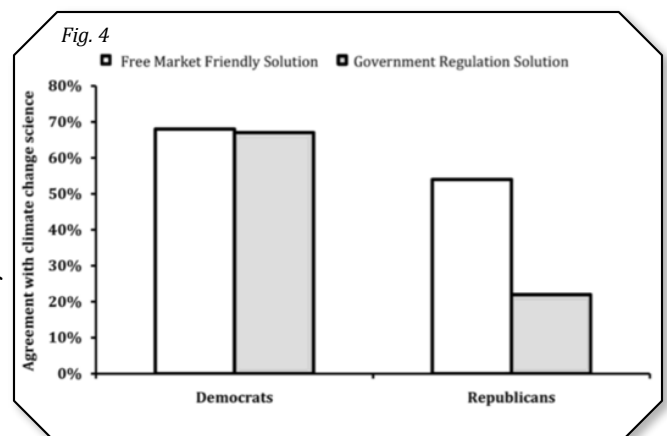
and ocean temperatures to rise, and as these temperatures rise, there will be visible consequences (Campbell et al., 2014). In a study conducted by Troy Campbell and Aron Kay, the authors hypothesised that a person's view on climate change has to with the political party they identify with, and that the potential solutions to climate change do not agree with Republican ideology. As a result, they predicted that Republicans do not necessarily deny that climate change is occuring, rather they are opposed to the steps that must be taken in order to halt climate change. The results of a survey that was conducted showed that Republicans showed increased skepticism to the solutions of climate change because of the way they feared these solutions could impact the economy. In a second survey, participants were first asked their political party, then read about a solution to climate change. The first solution was a regulatory emissions policy, which was expected be seem like an unappealing option to Republicans. The second was about a plan in which the United States could profit off of green energy. It was predicted that Democrats would equally approve of both solution plans. The results showed that Democrats were equalling accepting of both plans, whereas the Republicans were far more approving of the free market solution which involved the United States profiting off of green energy. It can be concluded that some people accept climate change as a real and pressing issue, however, express increased skepticism to the solutions (Campbell et al., 2014). By more openly discussing climate change as a universal issue, not a political one, there is more opportunity for agreement on solutions.

Reducing Household Carbon Emissions Using Behavioral Wedge

Most attention relating to solutions to climate change has been focused on long term options whose effects will take years to show. However, the issue is too pressing for only long term solutions to be implicated, and short term solutions are necessary until the effects of the long term solutions can be observed. Utilizing only near term solutions in US homes and nonbusiness travel, if nationally implemented, could save around 123 million metric tons of carbon each year by year ten. These short term solutions include weatherization updates, more efficient vehicles and vehicle adjustments, equipment maintenance and adjustments, and altercation to daily behaviors (Dietz et al,. 2009). Financial incentives, convenience features and strong social marketing could encourage the general population to adapt these environmentally beneficial behaviors. It can be accepted that if introduced properly, behavioral interventions can result in substantial benefits to the environment (Dietz et al., 2009).

Discussion

There are any different environmental threats that we as a human population are being forced to face. One very pressing issue is climate change. The Earth's atmosphere and oceans are heating up, which is impacting many different aspects of our ecosystems including the weather and wildlife. In order to halt climate change, it is necessary for humans to change the rate at which we are producing CO_2. There are different techniques being considered to help reduce the carbon footprint humans are leaving on the Earth. These techniques can be combined to help educate the public on their energy usage, and provide options to help them reduce their carbon usage. (Citations available upon request)

The Development of Social Essentialism in Children, by Lisbeth Hernandez, Sophomore

Essentialist beliefs about social categories have been found in early development by the age of 4 in every cultural context studied to date (Rhodes 2012). Essentialist beliefs about social categories such as race, gender, ethnicity, and religion correlate with increased stereotyping and more negative outgroup attitudes (Rhodes 2017). Studies have found that children ages 3-10 that essentialists beliefs about race predicted children's endorsement of negative racial stereotypes (Pauker 2010).

Psychological essentialism represents the intuitive theory of how certain critical aspects in our world are formed. This theory is a pervasive cognitive bias that leads individuals to view members of a certain category as sharing deep underlying, inherent essence (Rhodes 2012). Social Essentialism entails the belief that certain social categories (e.g, gender, race) mark fundamentally distinct kinds of people, and that each of those people have an underlying 'essence' (Rhodes 2012). Social

Fig. 2

Fig 1

essentialism emerges during the early stages of childhood when children attempt to make sense of their surroundings. Although, there is no doubt that social essentialism provides a scientifically inaccurate description of our environment. Essentialist beliefs are completely normal, although factors such as generic language and cultural context have a significant influence on the development of these beliefs about social categories.

Generic Language is a simple and ubiquitous way to communicate generalizations about categories, it refers to the abstract coherent kinds, instead of the specific individuals or subsets. During the early stages of development, generic language provides important cues that guide the acquisition of social categories (Rhodes 2017). This image shows "Zarpies'' an experiment used by Marjorie Rhodes in order to test whether hearing generic language induces social essentialism. Children and adults were shown a variety of these characters along with a line describing the depicted property which accompanied each page using a specific language depending on which condition the individual was placed in. The characters were diverse in terms of

sex, and race to ensure no previous essentialist beliefs about members of these certain categories would be applied to the characters. In this study, adults and children were separated and were placed into three conditions: generic (e. g "Look at this Zarpie! Zarpies are scared of ladybugs") specific (e.g "Look at this Zarpie! This Zarpie is scared of ladybugs") , and no-label (e.g "Look at this one! This one is scared of ladybugs"). The results are displayed in the adjacent graph (figure 1), indicating that those who were placed in the generic condition gave more essentialist responses than those in the other conditions such as specific and no-label. This study further supports the significance of how generic language leads to an increase in essentialist beliefs about a certain category. Social Essentialism develops slowly over time for children. The more generic language the child is exposed to when discussing social categories, the more likely they are to build on these essentialist beliefs. Cultural Context is a vital component in shaping the development of social essentialism. Cultural context focuses on the ideology, traditions, and values that surround and shape an individual's beliefs, behavior, decision-making, and opportunities. Cultural Context has been found to strongly shape whether children of various ages represent other social categories in essentialist terms (Rhodes 2017). Depending on where the child grows up can strongly impact the child's views in essentialist terms. A child who has been exposed to members of different social categories that they do not belong to, they hold more essentialist beliefs. African American children (who, on average, live in more diverse neighborhoods), develop essentialist beliefs about race at a younger age than White Children (Kinzler

2012).

Due to the scientifically inaccurate descriptions of essentialists beliefs, certain consequences such as prejudice, stereotyping and etc. arise when essence develops. Essentialism is necessary for such negative consequences to develop. If a child grows up constantly exposed to these negative attitudes at a young age, it influences them to act negatively towards those certain social categories and leading to implicit biases and negative group interactions.

A method that could possibly combat this issue is creating a program to teach adults in the education field to refrain from passing on personal essentialist beliefs. Perhaps teaching educators how to reduce the spread of personal opinions and biases that they have towards certain social categories could help reduce the amount of negative attitudes children develop towards certain social categories.

Social essentialism affects our everyday lives and how we view people. If we could get a better understanding of this intuitive theory and learn the consequences of it, we could also learn how to prevent it. Not many people know about the effects of social essentialism, our main goal should be educating and learning to keep more of an open mind about the accuracy of these beliefs, if we could learn to implement these ideas into our everyday lives, there could be a great reduction of negative inter-group relations and essentialists beliefs.

(Citations available upon request)

Fig. 3

The Physical and Psychological Effects of Placebos on the Brain, Olivia Albers, Sophomore

The placebo effect is a therapeutic treatment used to stimulate the effects of an active drug on the brain, body and behavior (Finess, 2011). There are many known effects of the placebo response that are often almost as or just as effective as traditional clinical treatments. The placebo effect is known to be a physiological phenomenon, and can even be distracting when looking at the effectiveness of active drugs (Mayberg, 2002). The difference between active drugs and a placebo is that active drugs are a form of analgesia which as some form of drug in it used for treatment, while a placebo is not a drug, but is made to mimic a drug and allow the patient's mind to have an effect on the body through psychological beliefs and expectations. Brain processes can be influenced by the intent and subjective thoughts in one's mind toward a form of analgesia. It has been proven before that having beliefs and expectations of what a drug (active or not) might do to the body can cause a neurochemical change in the brain involving the function of movement, pain, perception, and emotion processing in the body (Beauregard, n.d.).

Studies on the effect of placebo analgesia on the glucose metabolism of patients suffering from depression have been conducted to see if there is a more proactive way to treat this condition. Traditional active drugs could have harmful or irritating side effects on the user and the placebo effect might be the more effective option than the active drug overall, as well as take away the possibility of having side effects. The results of the study concluded that symptom remission was prominent in eight out of the fifteen patients that participated. Four of these patients had been treated with the active drug, and four received the placebo analgesia. The changes associated with the placebo effect in the responders that felt remission were regionally based in the brain. It was shown that the placebo will most likely have either an equal or greater magnitude of effectiveness as compared to an active drug in most patients. (Mayberg, et al., 2002)

The placebo effect was also studied in Parkinson's disease patients, to see if it can have an effect on the release of dopamine, a neurotransmitter involved in movement regulation, in the brain as a form of treatment. In Parkinson's disease (PD), dopamine is unable to follow the pathway of the brain from where it is released in the substantia nigra to where it is received in the striatum, causing the movement problems associated with the disease. Using the placebo, patients were tested to see if there can be a stimulation in the brain when there is an expectation of dopamine release after treatment, which can help patients where the neurons have not completely died yet. Using [C] raclopride (RAC) to compete with the dopamine to bind with receptors in the striatum, they measured the RAC binding potential before and after placebo and active drug treatment to see if there would be an effect. It was shown that the RAC binding potential decreased after both of the forms of treatment, and in some areas it was more prominent for the different forms of treatment, being active drug or placebo. The mean percent change in RAC binding after placebo administration shows the difference in how much dopamine was able to bind with the receptors, reaching over 15% in all regions tested. This shows that due to decrease in RAC binding, there was more dopamine in the striatum to bind with the receptors after treatment than before. This is furthers the point that because it shows that after the placebo was administered, the expectation of benefit in the patient cause a physical change in the brain that led to more endogenous dopamine being able to binding with the dopamine receptor, and helping to treat the patients (de la Fuente-Fernández, et al., 2001).

The rate of healing in duodenal ulcers using placebo analgesia was monitored over a four week period to see if the frequency of treatment had an effect on the effectiveness. Due to the treatment being a placebo, it would seem that the amount of times per day it was administered would not have an impact on how effective it was at treating the patient. There were two groups of patients, one receiving four doses of analgesia, and the latter receiving a two times a day regimen. After the four week

Site	Open baseline	Placebo	Mean percent change (range)
Head of caudate	1.964 ± 0.221	1.638 ± 0.230	16.6 (8.4–25.1)
Putamen			
Rostral	2.398 ± 0.342	1.976 ± 0.321	17.6 (5.3–26.3)
Intermediate	2.621 ± 0.438	2.142 ± 0.389	18.2 (7.4–27.0)
Caudal	2.095 ± 0.269	1.646 ± 0.261	21.2 (8.8–32.6)

Fig. 3 The RAC binding potential data is shown above, and is categorized by region of the brain and baseline vs. placebo, and the mean percent change. (Fuente-Fernández et al., 2001)

period ended, the four times a day regimen group had a 44% (805 out of 1821 patients), compared to the 32% (545 out of 1504 patients) that had a two times a day regimen. This data is significant because it illustrates that psychologically, the greater the patient's expectations are towards receiving remission due to a drug, or placebo, then the greater the effect will actually be. Administering a more frequent amount of doses will cause a patient to have a greater belief in the effectiveness of the drug, which then will make the treatment work more due to the placebo effect (de Craen, et al., 1999).

Most primary care physicians reported that 60-90 percent of their patients who complain about a common illness are actually suffering from stress or psychological factors that fabricated an illness and symptoms in their own mind. These patients should not receive traditional active drug treatment due to how there is no physical problem in the body, but by prescribing a placebo, the patient will receive the same magnitude of treatment. If the illness or problem was created in the mind, the mind is what is needed to be treated in order to fix it. Illnesses that are fabricated in the mind do not to be treated by an active drug, because a placebo will still have the same effect on the patient's mind, allowing it to recover.

It has been widely accepted that the placebo effect can provide treatment for 35% of people over a wide variety of diseases and disorders. There have been cure rates that have reached on average from 70%-100% from receiving baseline placebo analgesia. Endorphins, hormones secreted by the brain that affect psychological factors of thinking, such as movement, perception, pain and especially emotions. The levels of endorphins in the brain can be affected by placebos. This could mean that if endorphin levels change due to placebos, then endorphin levels in the brain could be the cause of physical changes of other hormones and neurotransmitters attributed to the placebo effect.

(Citations available upon request)

The Effects of Pollution on the Ocean Environment and Inhabitants, by Ava Bisordi-Potter, Sophomore

There are many different factors when it comes to pollution and there are many different types of pollution. All of them have the similar consequences, and it is damaging the ocean environment and its inhabitants.

Copper Effects

Oceans all around the world are rapidly accumulating with pollution. Effects from copper pollution is making the results of ocean acidification and warming even worse on the early life stages of kelp in the oceans. The lifecycle completion of the kelp is disrupted by the copper pollution and it prevents it to continue and complete its process. Macrocystis pyrifera and Undaria pinnatifida, are the two species of kelp that were exposed to factorial combinations of conditions of current and 2100-predicted temperature, pH and two copper levels for a certain amount of days. Their meiospore germination declined in a range from 5-18 percent with all combinations tested, excluding under ambient temperature and current pH level. Next the growth rate of germlings produced by the kelp species was studied. Ocean warming and acidification greatly affected the growth rate of germlings for the Macrocystis pyrifera species. On the contrary, ocean warming and acidification had no effect on the growth rate of Undaria pinnatifida germlings. Furthermore, with all combinations tested with the effect of copper, copper caused a significant reduction in the germling growth rate for both species, ranging anywhere from a 46-68 percent decrease. On the fifteenth day being under No-Cu treatment, both species experienced sexual differentiation of gametophytes. The macrocystis pyrifera species had an 18 percent increase of gametophyte size for males, while females had a 46 percent increase. Only the Undaria pinnatifida species had a 24 percent reduction in females. The gametophyte sex ratio was not remarkably affected by single factors or their interactions. In cultures of both species where copper was exposed, copper- binding ligand concentrations were higher. Results showed that copper pollution is a more notable factor than global climate drivers when controlling the meiospore development in kelp because of the effects it has on the completion of their life cycles (Leal et al., 2018).

Long Term Effects

The development of quick growing coastal regions are dealing with the repercussions of different land uses and climate change. Some of these include flooding and an increase in sediment, chemical and nutrient runoff. This could lead to the increase of pathogen runoff. Pathogen runoff is a serious danger to the health of animals, people and their ecosystems. The spatial distribution of parasite runoff, precipitation, and development effects on projected pathogen delivery to the ocean is studied. Oocysts of Toxoplasma gondii

Fig 1

Human production and activities are affecting the quality of water. It is very hard to identify the radius of surface water pollution. In the Jinghe Oasis, the studying of impacts of land use/cover on surface water pollution was by the use of remote sensing and three-dimensional fluorescence technologies. Specifically, self-organizing map (SOM) and the PARAFAC model were used. The PARAFAC model was able to successfully remove four fluorescence components, microbial humic-like (C1), terrestrial humic-like organic (C2, C4), and protein-like organic (C3) substances. Five buffer zones were built from thirty water sampling points. Any important correlation between land use and fluorescence components were all within a two hundred meter buffer. Urban and salinized sources on land

that are found in the feces of both domestic and wild felids are being transported by freshwater runoffs into the ocean. The 300-fold difference in complete projected oocyst delivery from land to sea between watersheds was found. Watersheds with greater human development were found to contain a greater amount of large parasite loads than watersheds not affected by humans. They align comparatively with regions with increased sentinel marine mammal T. gondii infection. While the majority of T. gondii transportation along parts of the coast is due to wild felids, greater developed areas with larger domestic cat populations are contributing greatly as seen in figure 1. Thus showing the impact they could have in land-sea pathogen flow. Even though the T. gondii infection was higher in sampled wild felids in southern California and central coastal California, domestic cats most likely have a greater contribution to coastal environments with higher amounts of oocysts. Precipitation and coastal developments increased the oocysts transportation to the ocean averaging 44 and 79 percent increases and parasite runoff combined had an 175 percent increase. Anthropogenic changes to the climate and landscape of a region can increase the runoff of diverse pathogens from the land to sea which better allows for the transference

to wildlife, humans and animals (VanWormer et al., 2016).

Fig. 2

were main pollution contributors. The data collected how the interrelation of salinized land, cropland and the fluorescence peaks of C1, W2, and W7 were consequential by the method of stepwise multiple regression (Wang and Zhang, 2018).

Overtime, trash has accumulated in the ocean in certain areas. The annual worldwide plastic consumption has passed 320 million tonnes. They characterise and quantify a large part of subtropical waters where plastic has become quite abundant in the region that is named the Great Pacific Garbage Patch. Based on the type of debris that was collected, small surface-to-volume ratio, it displayed how only certain types of plastics can last and accumulate at the surface of this region. Microplastics in the area accounted for 8 percent of total mass of debris but 94 percent of the estimated 1.8 (1.1–3.6) trillion pieces found floating on the surface. More than three-quarters of the mass was carried by debris larger than 5 cm and they found that at least 46 percent of it was made up of fishing nets. Overall results displayed in Figure 2 show how plastic pollution is rapidly increasing at an alarming rate in that area. Especially when compared to surrounding regions. Which could has a great to not only the ocean everything is accumulating in, but areas where debris can end up at (Lebreton et al., 2018).

Seventyone percent of the coastlines in the world are warming at an alarming rate. The coastal ecosystems are affected the most out of all the marine systems. Overpopulation, agricultural runoff and pollution along with human influences are contributing to the causes of the warmings. The majority of the coastlines studied showed a rapid increase of temperatures warming compared to past years long ago, observed between the Tropic of Cancer and the Arctic Circle. Intense warming rates were also observed in open coasts such as off Eastern China, Western Africa or Northeast South-America. Many studies on the climate have shown the general trend of increasing temperatures of the sea and land surfaces. Global warming assessments do not provide the specific details of the seasonality of changes, but with the new advancements in technology, the ability to analyze local patterns within the global context has been achieved. This will furthermore be an advantage to a wide range of scientific fields of study, allowing for the continuation of studies on the impact these factors are having on the environment (Lima and Wethey, 2012)

(Citations available upon request)

FUN TIMES

What happens in Science Research stays in Science Research
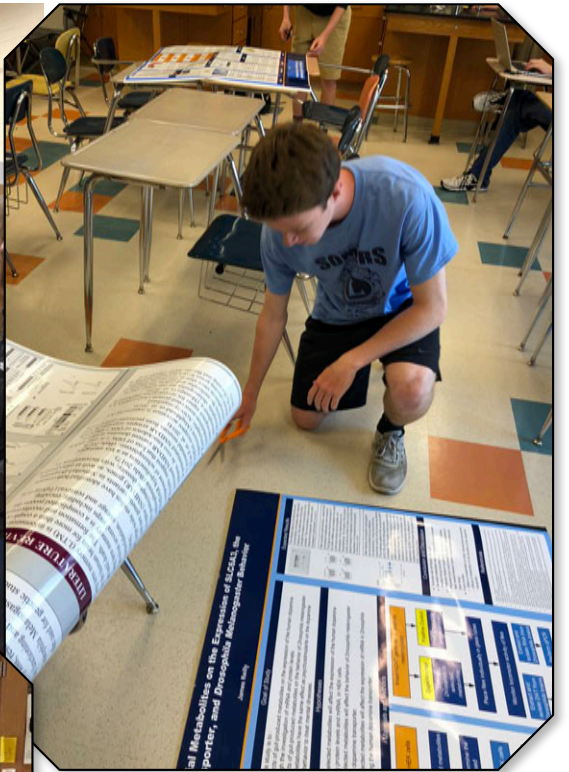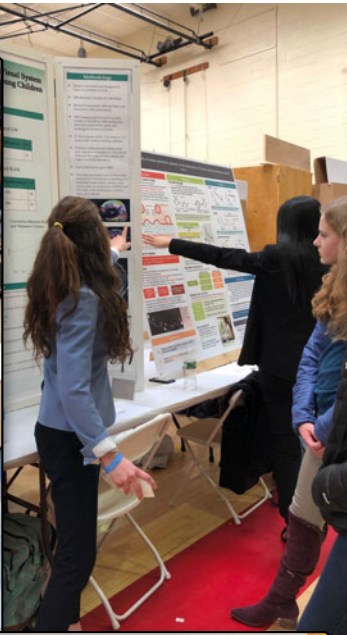
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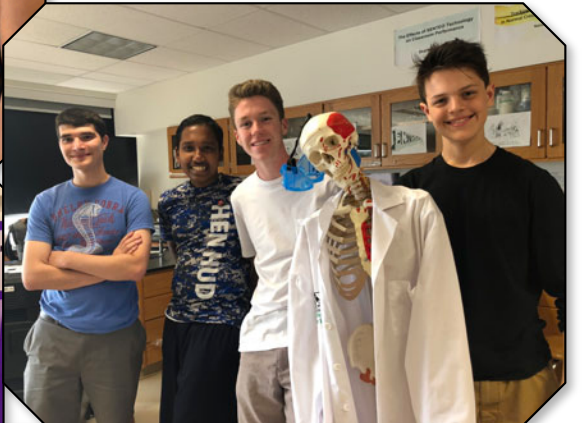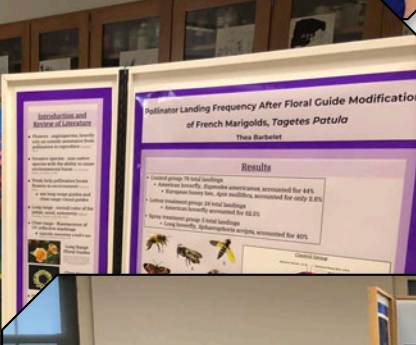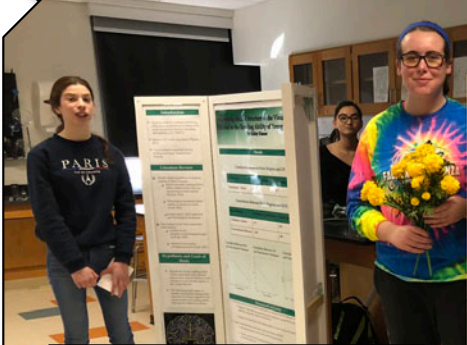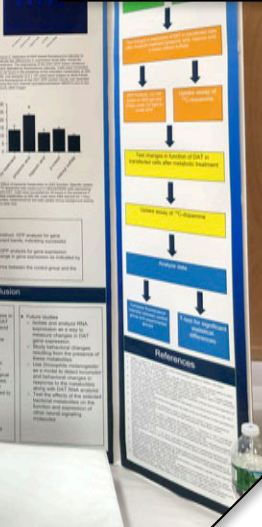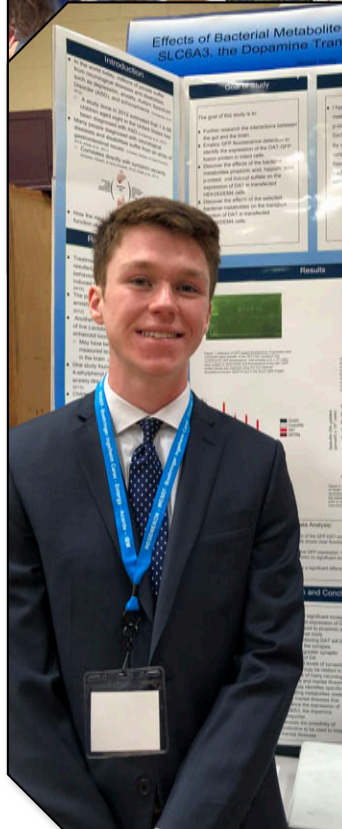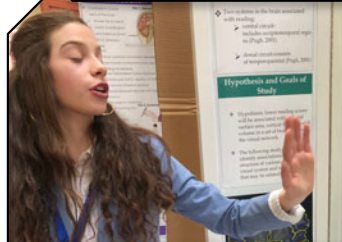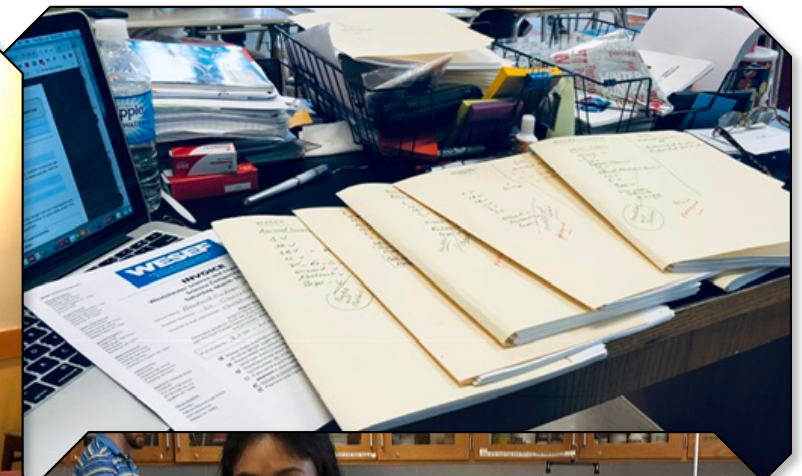
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