Hendrick Hudson
High School

Science Research

May 2017

Sponsored by the Hendrick Hudson Community Educational Foundation (HHCEF)

" You are limited only by your imagination"

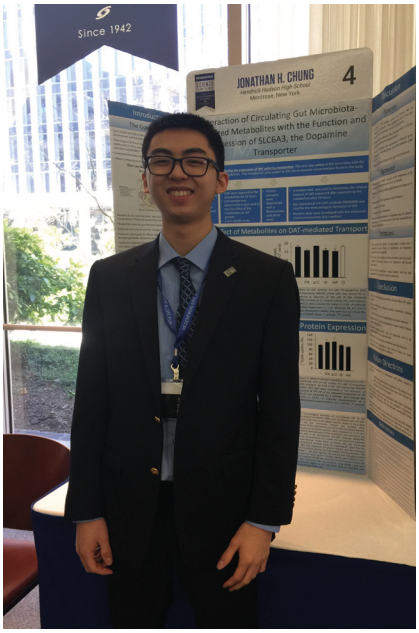
Hendrick Hudson High School

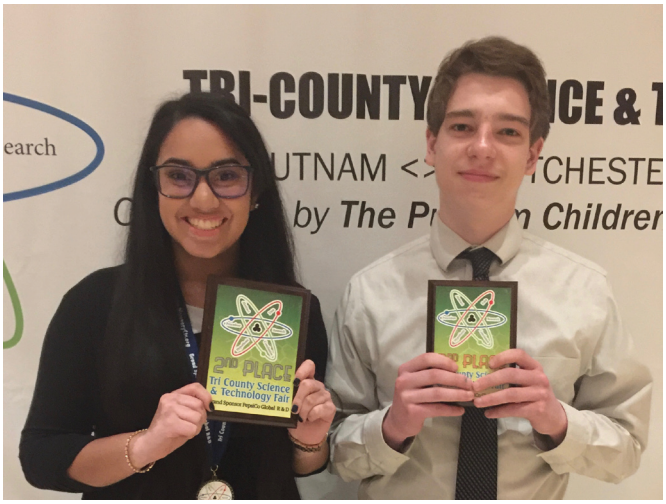
May 2017

Science Research

The Hendrick Hudson Science Research program had a very successful year. Jonathan Chung has been named a finalist in the Regeneron Science Talent Search, the nation's oldest and most prestigious science and math competition for high school seniors. He also received science report and student initiative badges for his paper submission. Melanie Porras has been named a scholar (semi-finalist) for her project on artificial photosynthesis. 300 semi-finalists and 40 finalists are selected from a pool of over 1700 applicants. The Regeneron Science Talent Search, a program of Society for Science & the Public, focuses on identifying the next generation of scientists and engineers who will provide critical leadership in solving some of the world's most pressing challenges while shaping the future of research and development for our nation and the world. Jonathan Chung presented his project in Washington, D.C., where he also had the opportunity to meet with national leaders and share their projects with the public at the National Geographic Society.

Matthew Gofman received science report and student initiative badges for his submission to the STS competition and placed third at the Tri-County Science Fair. Melanie Porras placed First in the plant sciences category at the Westchester Science and Engineering Fair (WESEF). Isabella Brizzi received the Stockholm Water Prize at WESEF as well. Melody Munitz competed as a junior at WESEF and got third place in the Behavior category. Deepa Mistry placed second at the Tri-County Science Fair.





Interaction of Circulating Gut Microbiota-generated Metabolites with the Function and Expression of SLC6A3, the Dopamine Transporter, by Jonathan Chung (STS FINALIST, paper excerpt) (Senior)

Abstract

Many mental conditions show comorbidity with gastrointestinal disorders. Circulating metabolites from microorganisms in the gut have been documented to be involved in communication between the gut and brain. This study tests the effects of four representative metabolites (propionic acid, indoxyl sulfate, hippuric acid, and p-cresol) on the activity of one key target involved in neurotransmission, the dopamine transporter (DAT). It was hypothesized that these four metabolites would affect the function and/or expression of DAT. Using a plasmid that carries the recombinant DAT gene, a recombinant bacmid was created to infect attached HEK293-EM4 cells to assess the transport activity and expression of DAT in response to exposure to these metabolites. Uptake assays with 14C-dopamine as substrate and the 4 metabolites at 100 μM and 500 μM revealed that at 100 μM, propionic acid, hippuric acid, and indoxyl sulfate reduced 14C-dopamine uptake. Meanwhile, p-cresol did not significantly affect uptake at 100 μM. All four metabolites had little effect on 14C-dopamine uptake at 500 μM. To test expression, HEK cells were exposed to the metabolites for 24 hours at 250 nM. A bicinchoninic acid assay, western blot, and uptake assay revealed that exposure to p-cresol and propionic acid increased the expression of DAT, while exposure to indoxyl sulfate and hippuric acid reduced it. Altered DAT expression in the brain translates to an altered synapse clearance rate of dopamine and altered dopaminergic signaling, both of which have relevance to many diseases implicated in gut-brain communication, such as autism and schizophrenia. It was concluded that circulating metabolites play important roles in the gut-brain axis.

Introduction

The Gut-Microbiota-Brain Axis

The gut microbiota comprises trillions of microbes that influence gastrointestinal physiology as well as the function of distant organs and susceptibility of the host to disease (Evrenel and Ceylan, 2015). The brain is one such organ as the gut microbiota regulates the development and function of the brain through the gut-brain axis, a bidirectional means of communication between the central and the enteric nervous systems (Carabotti et al., 2015; Collins et al., 2012; Dinan and Cryan 2013). In support of the gut-brain axis, many neurological diseases such as Autism, Rett syndrome, Cerebral Palsy, Schizophrenia, and major depression report gastrointestinal issues as a prevalent comorbidity (Heijtz et al., 2011). For example, a study that included over 14,000 individuals with Autism Spectrum Disorder (ASD) found a higher prevalence of inflammatory bowel disease (IBD) and other GI disorders in ASD patients compared to controls (Kohane et al., 2012). Studies have also reported that gastrointestinal disorders correlate with symptom severity in ASD (Coury et al., 2012; Buie et al., 2010). While the causes of such disorders are insufficiently understood, they may be linked, directly or indirectly, to gut bacteria as ASD individuals exhibit an altered composition of the intestinal microbiota (Kang et al., 2013; Finegold et al., 2010; Gondalia et al., 2012; Finegold et al., 2002). However, a definitive answer as to how the gut and brain communicate, whether through neural, endocrine, metabolic or immune pathways, is unclear (Zhang and Davies, 2016).

Modulation of the Brain and Behavior through Probiotics

Many studies have used probiotics, live bacteria or bacterial products, to alter the brain and behavior (Liu et al., 2016). For example, the probiotic Lactobacillus rhamnosus decreased anxiety and despair-like behavior and reduced stress-induced increase of plasma corticosterone levels in mice (Cryan & Dinan 2012). Meanwhile, Bifidobacteria treatment reduced levels of 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex and decreased levels of 3,4-dihydroxyphenylacetic acid (DOPAC) in the amygdaloid cortex (Desbonnet et al., 2010). Another study demonstrated that chronic L. helveticus NS8 supplementation can counteract, with the efficacy of the selective serotonin reuptake inhibitor citalopram, chronic stress-induced behavioral, cognitive, and biochemical aberrations (Liang et al., 2015). Live Lactobacillus plantarum PS128 treatment also affected biochemical aberra-

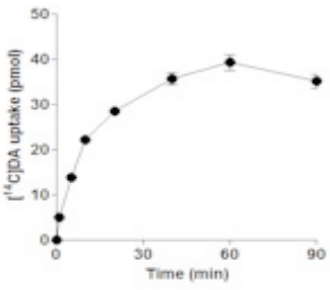


Fig. 2. ^{14}C-dopamine uptake from 0 to 90 minutes is represented in picomoles per $1.25x10^5$ cells.

tions, significantly increasing the levels of dopamine and serotonin in the brain. The enhancement in striatal dopamine neurotransmission following probiotic treatment may have caused the enhanced locomotor activity observed (Liang et al., 2015). These successes with probiotics support the existence of the gut-brain axis. But more importantly, the ability of probiotics to alter monoamine levels in the brain and normalize both the gut microbiota and behavior reveal the potential role of bacteria in regulating gut-brain communication.

Bacterial Metabolites as Messengers in the Gut-Brain Axis

One method of communication between the gut and the brain is through bacterial metabolites, which are the intermediates and end points of biological processes (Hsiao et al., 2013). Different bacteria produce different metabolites meaning that an altered gut microbiota creates a different circulating metabolite profile (Dinan et al., 2015). The Maternal Immune Activation (MIA) model yields mice that exhibit core behavioral symptoms of ASD, a common autism neuropathology, increased gut permeability, tight junction defects, and dysbiosis, an impaired microbiota. In these mice, eight percent of all serum metabolites were significantly altered. The metabolite 4-ethylphenylsulfate (4EPS) was of particular interest due to a staggering 46-fold increase in serum levels of MIA offspring that was completely restored by probiotic B. fragilis treatment. Indolepyruvate, a key molecule of the tryptophan metabolism pathway, also had elevated levels in serum. Notably, the commensal bacteria modulated, if not directly produced, both indolepyruvate and 4-ethylphenylsulfate. Given its potential role in behaviors relevant to ASD, 4-EPS was tested separately on naive mice. Mice treated with 4EPS potassium salt from 3 weeks of age to 6 weeks of age displayed anxiety-like behavior similar to that observed in MIA offspring. This outcome suggests that specific symptoms relevant to ASD and other neurodevelopmental disorders may be connected to specific metabolites. Combinations of metabolites may control more complex behaviors (Hsiao et al., 2013). In the gut, metabolites can influence neurotransmitter production. Metabolites upregulated by spore forming bacteria, such as α-tocopherol, tyramine, and 4-aminobenzoic acid (PABA), have been shown to promote 5-hydroxytryptamine (5-HT, serotonin) biosynthesis both in vivo, through injecting short chain fatty acids in GF mice, and in vitro, through testing short chain fatty acids on chromaffin cell cultures (Yano et al., 2015; Nozawa et al., 2009). These metabolites increased tryptophan hydroxylase 1 (TPH1) expression, suggesting that colonic enterochromaffin cells receive signals from these particular metabolites to enhance 5-HT biosynthesis. In germ-free mice, the elevated luminal concentrations of particular microbial metabolites increased colonic and blood 5-HT levels. The increased 5-HT levels increased 5-HT uptake by circulating platelets. The indigenous microbiota, which produces many of these metabolites, can even modulate hippocampal levels of 5-HT, indicated by

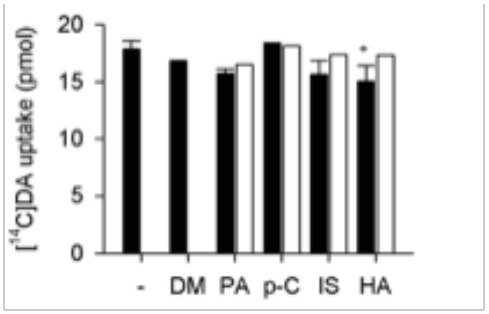


Fig. 3. ^{14}C -dopamine uptake was tested at 5 minutes at metabolite concentrations of 100 µM and 500 µM. The black bars represent uptake at 100 µM of the indicated compounds and the open bars show the uptake in the presence of 500 µM of the indicated compounds. The error bar is S.E.M. The DMSO condition [DM] was done only once and uptake at 500 µM were single determinations so there are no error bars for these conditions. Hippuric acid [HA] was dissolved in DMSO. All other metabolites (propionic acid [PA], p-cresol [p-C], indoxyl sulfate [IS]) were dissolved in water. The "-" group is the control. *, $p<0.05$. **, $p<0.01$.

disruptions in the serotonergic system in the absence of a normal gut microbiota (Clarke et al., 2013). Other metabolites such as 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), a Clostridia metabolite, inhibit dopamine beta hydroxylase (Shaw, 2010). HPHPA among others were found at significantly higher concentrations in the urine samples of children with autism and in schizophrenia. The inhibition of dopamine beta hydroxylase, which is required for the conversion of dopamine to norepinephrine, may account for the excess dopamine implicated in the etiology of psychotic behavior and schizophrenia (Shaw, 2010).

Metabolites on Brain Neurotransmission

Metabolites affect the brain directly. Propionic acid, an enteric bacterial metabolite, reaches the brain by crossing the gut-blood and blood-brain barrier passively and actively via high affinity transporters (Thomas et al., 2012; Conn et al., 1983). Concentrating intracellularly in the brain, propionic acid deleteriously affects various neurophysiological processes, including neurotransmitter release, gene expression, mitochondrial function, immune modulation, gap junction gating, and behavior (DeCastro et al., 2005; Maurer et al., 2004). The increased intracellular neuronal and glial acidification and calcium proportions altered neurotransmitter release, which included glutamate, dopamine, norepinephrine, and serotonin (El-Ensary et al., 2012). These observations following propionic acid infusions demonstrate the ability of metabolites to affect neurotransmitter signaling. As part of investigating the effects of antibiotic treatment on cognitive impairment, a recent study recorded differences in the expression of neural signaling-related molecules in the brain (Frohlich et al., 2016). Circulating metabolite levels were also examined after antibiotic treatment. Many metabolites, including p-cresyl sulfate, propionate, deoxycholic acid, and trimethylamine-N-oxide, had markedly different levels following antibiotic treatment. The serotonin transporter, SLC6A4, was among the tested neural signaling-related molecules in the brain. After antibiotic treatment and when compared to the vehicle-treated mice, SLC6A4 mRNA expres-

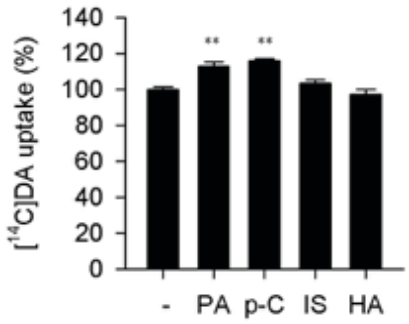


Fig. 4. ^{14}C -dopamine uptake was performed with HEK293-EM4 cells exposed to the virus, NaB, and the indicated metabolites at 250 nM for 24 hours. Triplicates were done for each group (n=3). Uptake was measured at the 5-minute time point and data are shown as percentage of untreated cells (-). Abbreviations were consistent with those in the legend to Figure 3. *, p<0.05. **, p<0.01

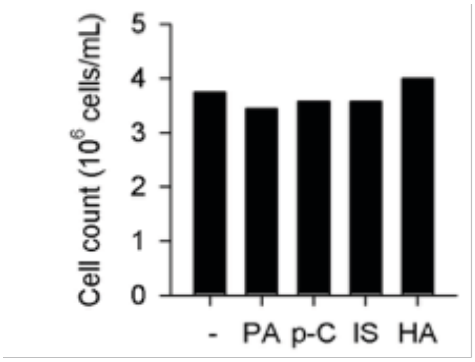


Fig. 5. After 24 hours of exposure to the metabolites at 250 nM, HEK293-EM4 cells were detached from the wells and counted under a microscope. All 4 metabolites had no significant effect on cell growth. Abbreviations were consistent with those in the legend to Figure 3.

sion was slightly reduced in the hypothalamus and hippocampus, while mRNA expression was increased in the medial prefrontal cortex and significantly increased in the amygdala. It was speculated that the circulating metabolites might be responsible for gut-brain axis communication.

Statement of Purpose

Previous studies have shown that circulating bacterial metabolites may have an integral role in communication between the gut and brain because of the markedly altered metabolite profiles recorded in mental illnesses and dysfunction. Separately, metabolites have been shown to affect neurotransmission and behavior. Monoamines and their transporters have been implicated in the gut brain communication by probiotics altering levels of dopamine and serotonin in the brain and producing similar effects to the selective serotonin reuptake inhibitor citalopram. Additionally, antibiotic-induced cognitive impairment altered the levels of many circulating metabolites, which led to alterations in the levels of expression of the serotonin transporter. However, to my knowledge (after intensive literature research and consultation with my mentor) no study has tested the effects of metabolites on DAT at the molecular level. Given the identification of several circulating metabolites that had significantly altered levels in a variety of mental conditions, it was hypothesized that these metabolites could interact with the function and expression of DAT. Four metabolites, propionic acid, p-cresol, hippuric acid, and indoxyl sulfate, were chosen for the study. To test these metabolites on a molecular level with function and expression, uptake of 14C-dopamine and DAT expression in attached HEK293-EM4 cells were both performed in the presence or absence of these metabolites. As an independent research project, the study was conceived by me, the student, and was not part of ongoing work in my mentor's lab.

(...)

Results

Fig. 2 confirms the successful expression and function of DAT in the HEK cells using the recombinant bacmid virus. The 14C –dopamine uptake curve from 0 to 90 minutes was used to choose the 5-minute time point for uptake in the presence of the metabolites. Fig. 3 shows that overall uptake of 14C -dopamine was not significantly affected regardless of whether the assay was at metabolite concentrations of 100 nM or 500 nM. At the 5-minute time point for both concentrations, the uptake, measured in picomoles, remained between 15 and 20 picomoles. Uptake was slightly reduced in the presence of propionic acid (PA) at 100 μM and indoxyl sulfate (IS) at 100 μM. Uptake in the presence of DMSO was also slightly reduced, which may account for the lower uptake in the presence of hippuric acid (HA), the only metabolite dissolved in DMSO, at 100 μM. Although uptake was slightly reduced for several groups at 100 μM, uptake did not dramatically change when the concentration of the metabolites was increased to 500 μM. This disproved the hypothesis that these metabolites may directly interact with the function of DAT by inhibiting uptake or some other mechanism.

The uptake assay performed with the cells exposed to the metabolites at 250 nM for 24 hours reflected greater variations in the uptake of 14C-dopamine. Fig. 4 indicates that the uptake for the p-cresol group was roughly 20 percent greater than that of the control. The propionic acid group also showed about a 15 percent increase in uptake compared to the control. The increased uptake indicates that more DAT was expressed and thus more was present to uptake 14C -dopamine. The observable changes in expression in this uptake assay align with the fold changes in SLC6A4 mRNA expression observed in mice with antibiotic-induced dysbiosis compared to vehicle-treated groups (Frohlich et al., 2016). In that study, the fold changes in expression of SLC6A4 in the medial prefrontal cortex, hippocampus, amygdala, and hypothalamus never left the range of 0.5 to 1.5.

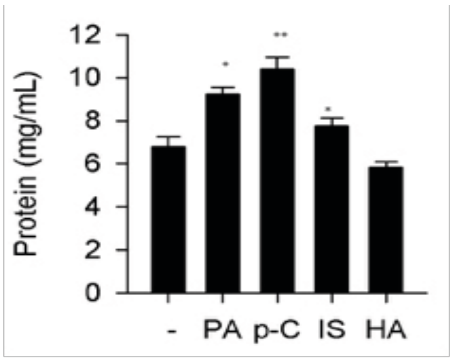


Fig. 6. Determination of the BCA was done in triplicate (n=3). The error bar represents S.E.M. Abbreviations were consistent with those in the legend to Figure 3. *, p<0.05. **, p<0.01

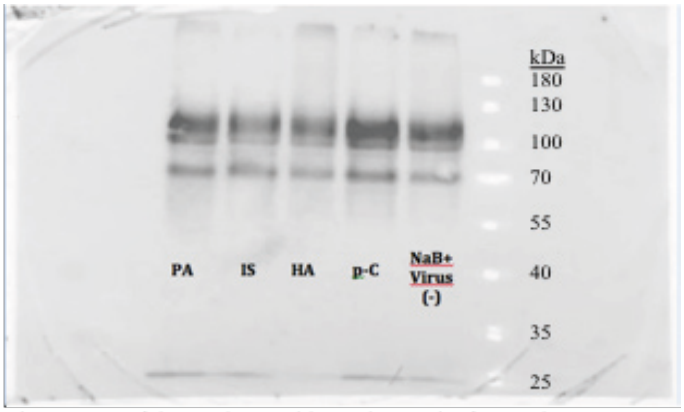


Fig. 7. Image of the membrane with protein samples from each group. The far right lane represents the marker and the molecular weight (in kilodalton, kDa) is indicated. The upper band (~ 100 kDa) signifies the mature and fully-glycosylated DAT and the lower band (~ 70 kDa) signifies immature (non-glycosylated) DAT.

However, the percent change in uptake for the IS and HA groups were less

Fig. 5 shows that the 4 metabolites were not toxic to the cells and did not affect cell growth. Cell counting revealed cell concentrations from ~3.75 to 4 million cells/ml for all groups. This indicated that any changes in the amount of DAT expressed would be attributable to a direct effect on expression rather than on cell growth. The results of the bichinchoninic acid assay (BCA) assay showed significant differences in protein concentrations for the different metabolites after exposure for 24 hours. Compared to the control group that received only sodium butyrate and the virus, protein concentrations were elevated for the groups cultured in propionic acid, indoxyl sulfate, and p-cresol. The propionic acid and p-cresol groups in particular displayed large differences in protein concentration. Meanwhile, the protein concentration of the cells cultured with hippuric acid was only slightly reduced compared to the control.

A western blot was performed to test specifically for the expression of DAT. Fig. 7 reaffirms the results of the BCA assay. The darker bands indicate that more DAT is present, which means more DAT was expressed. There are two bands for each group because of the presence of both immature and mature proteins. The immature proteins, represented by the lower bands, are smaller than the mature proteins and thus traveled farther. The amount of immature and mature proteins had a positive correlation in all groups. Both the upper and lower bands for DAT in the presence of p-cresol were much darker than the control group, indicating increased expression of DAT. The increased expression was consistent with the elevated protein concentrations obtained from the BCA assay. For propionic acid, the upper and lower bands were also darker than the control, but not as pronounced as those of p-cresol. As with p-cresol, the increased expression matched the higher protein concentrations obtained from the BCA assay. Like the BCA, expression of DAT seemed to be higher in p-cresol than in propionic acid. On the other hand, the cells exposed to indoxyl sulfate and hippuric acid exhibited decreased expression of DAT. The lighter bands for both indoxyl sulfate and hippuric acid reflected the decreased expression. The altered expression levels of DAT support the hypothesis that these metabolites could alter the expression of DAT.

Discussion

This was the first study to test the effects of circulating bacterial metabolites on the dopamine transporter at a molecular level. In a previous study, antibiotic-induced gut dysbiosis changed the profile of circulating metabolites, including propionate and p-cresyl sulfate (Frohlich et al., 2016). These metabolites are similar to propionic acid and p-cresol, metabolites used in this study. The results matched the conclusions of this previous study, which concluded that cognitive impairment by antibiotic-induced gut dysbiosis caused changes in the expression of SLC6A4, the serotonin transporter (Frohlich et al., 2016). However, this study tested the effects on the function and levels of expression of DAT in response to these metabolites and is unprecedented in the literature. The altered uptake after 24 hours of exposure at 250 nM, altered protein concentrations, and differences in band darkness in the western blot can be attributed to expression because cell growth was confirmed to be unaffected by the metabolites. Similarly to how SLC6A4 may be involved in the cognitive deficits in previous studies, altered expression of DAT has implications in many mental illnesses related to the gut-brain axis. The significant increased expression of DAT by p-cresol and propionic acid indicates that more DAT would be present in the brain. This means that there would be an increased synapse clearance rate of dopamine by the re-uptake of the transporter. The decreased extracellular concentrations of dopamine may be related to the etiologies of ASD, depression, anxiety, and other mental disorders relevant to the gut-brain axis. This altered dopaminergic signaling has implications to schizophrenia, which has been found to reduce DAT expression in the amygdala (Markota et al., 2014). Hippuric acid and indoxyl sulfate could be relevant to this observation as these metabolites decreased DAT expression in this study. The amygdala was also the brain region that showed the most noticeable differences in expression of SLC6A4 after antibiotic treatment (Frohlich et al., 2016). The commonality elucidates a role for circulating metabolites to cause mental conditions such as cognitive impairment or schizophrenia by altering the expression of monoamine transporters.

My study identifies specific metabolites that significantly influence neurotransmission by altering the expression of neural-signalling molecules. Identification of these metabolites reveals more efficient probiotic and antibiotic treatments. The use of proper probiotics and antibiotics can change the gut microbiota composition towards one that does not produce bacterial metabolites harmful to the brain. Specifically, my research suggests that the use of probiotics to normalize the amount of bacteria that produce propionic acid and p-cresol would normalize monoaminergic signalling in the brain.

These results expand upon the work of previous studies that show that propionic acid affects neuronal communication, behavior, and neurotransmitter levels, including dopamine and serotonin (El-Ansary et al., 2012).

Altered expression of DAT and SERT by circulating metabolites could be responsible for the altered levels of dopamine and serotonin after propionic acid injections. While these metabolites did not directly affect the function of DAT, propionic acid may affect the function of DAT indirectly by altering brain phospholipid molecular species (Thomas et al., 2012). Specifically, propionic acid increased the accumulation of molecular species with arachidonic acid and decreased the proportions of molecular species with docosahexaenoic acid (Thomas et al., 2012). Arachidonic acid has been found to inhibit dopamine uptake by DAT (Chen et al., 2003). The other 3 metabolites may function in a similar fashion by altering phospholipid molecular species, which may in turn influence DAT function and uptake. A potential implication of dopamine reuptake in gut dysbiosis-mediated alterations in brain function can be assumed from the altered expression of DAT by the 4 metabolites. Propionic acid has been used to create animal mouse models of autism (Shultz et al., 2008). Given the altered dopamine signaling in autism, the effects of propionic acid on the expression of DAT may be related to the etiology of autism. Taking into account all the data with circulating metabolites, it is evident that gut dysbiosis-related changes in the concentrations of circulating molecules may be a means to signal to the brain and modify its function.

Conclusions and Future Work

This was the first study of this kind on DAT. Developed and conducted by me, the student, this independent project was entirely original and not part of ongoing work in the mentor's lab. In this study, the four selected circulating metabolites, propionic acid, indoxyl sulfate, hippuric acid, and p-cresol, did not affect DAT function, as shown by no substantial effect on uptake at 5 minutes at both 100 μM and 500 μM. However, the four metabolites, when added at 250 nM to cells expressing DAT, did influence DAT expression, as evidenced by the BCA assay, western blot, and uptake assay. Propionic acid and p-cresol increased DAT expression while hippuric acid and indoxyl sulfate reduced expression. From cell counting it was determined that the difference in expression was not due to metabolite cell toxicity or effects on cell growth. These results warrant a future study with a larger sample size, allowing for a more thorough analysis of the effect of the representative compounds in this study. The altered levels of expression corroborate recent findings that circulating metabolites may be involved in altering the expression of SLC6A4, the serotonin transporter, in various brain regions, most notably the amygdala (Frohlich et al., 2016). They also corroborate other studies that reference changes in monoamine levels and neurotransmitter signaling in the brain (Liu et al., 2016). These results at the molecular level also confirm the hypothesis that profound alterations in the circulating metabolite profile may be relevant to the communication between the gut and brain. However, as the circulating metabolite profile is much more complex than 4 metabolites, the systemic effects of metabolites on monoamine transporters are difficult to quantify. This study only tested the effects of the metabolites that were readily available from commercial sources on expression and function individually. In the body, these metabolites would interact with the brain simultaneously and under more complex conditions. In the future, I would like to replicate the results of this study and look further into the expression by examining the mRNA of DAT. I would like to look into the mRNA transcription factors involved in the transcription of DAT mRNA and other mechanisms that may have altered the expression of DAT. Future studies could test these metabolites with other implicated neural-signaling molecules such as the serotonin transporter, norepinephrine transporter, brain-derived neurotrophic factor (BDNF), and the N-methyl-D-aspartate receptor subunit GRIN2B. These findings contribute to the understanding of the microbiota-gut-brain axis and explore causality in the interaction between the gut microbiota and brain. By understanding the effects of circulating enteric-derived bacterial metabolites on the brain, more can be revealed about the mechanism of communication of the gut-brain axis. These findings about the effects of metabolites on monoamine transporters provide insight into how circulating metabolites influence mental illnesses, cognitive function, and behavior at the molecular level.

Mentor: Dr. Matthias Quick, Ph.D.

ASSISTANT PROFESSOR of Clinical Neurobiology, Department of Psychiatry and Center for Molecular Recognition,

Columbia University and College of Physicians and Surgeons.

We are very grateful for the dedication and help of Dr. Quick.

Bibliography

Buie T, Campbell DB, Fuchs GJ, 3rd, Furuta GT, Levy J, Vandewater J, Whitaker AH, Atkins D, Bauman ML, Beaudet AL, et al. Evaluation, diagnosis, and treatment of gastrointestinal disorders in individuals with ASDs: a consensus report. Pediatrics. 2010;125(Suppl 1):S1–18.

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. Annals of Gastroenterology : Quarterly Publication of the Hellenic Society ofGastroenterology,28(2), 203–209.

Chen N, Appell M, Berfield JL, Reith MEA. Inhibition by arachidonic acid and other fatty acids of dopamine uptake at the human dopamine transporter. Eur J Pharmacol. 2003;478:89–95. doi: 10.1016/j.ejphar.2003.08.045.

Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, Dinan TG, Cryan JF. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry. 2013;18:666–673.

Collins SM, Surette M, Bercik P. The interplay between the intestinal microbiota and the brain. Nat Rev Microbiol 2012; 10: 735–742.

Conn AR, Fell DI, Steele RD: Characterization of alpha-keto acid transport across blood brain barrier in rats. Am J Physiol 1983, 245:E253-E260.

Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, Kaul A, Mawe G, Patterson P, Jones NE. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. Pediatrics. 2012;130(Suppl 2):S160–168.

Cryan JF, Dinan TG (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 13:701–712.

DeCastro M, Nankova BB, Shah P, Patel P, Mally PV, Mishra R, La Gamma EF: Short chain fatty acids regulate tyrosine hydroxylase gene expression through a cAMP dependent signaling pathway. Brain Res Mol Brain Res 2005, 142:28–38.

Desbonnet L., Garrett L., Clarke G., Kiely B., Cryan J.F., Dinan T.G. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience. 2010;170:1179–1188. doi: 10.1016/j.neuroscience.2010.08.005.

Dinan, T. G. and Cryan, J. F. (2013), Melancholic microbes: a link between gut microbiota and depression?. Neurogastroenterol. Motil., 25: 713–719. doi:10.1111/nmo.12198.

Dinan TG, Stilling RM, Stanton C, Cryan JF.. Collective unconscious: how gut microbes shape human behavior. J Psychiatr Res (2015) 63:1 9.10.1016/j.jpsychires.2015.02.021.

El-Ansary, A.K.; Ben Bacha, A.; Kotb, M. Etiology of autistic features: The persisting neurotoxic effects of propionic acid. J. Neuroinflamm. 2012, 9, 74.

Evrensel, A., & Ceylan, M. E. (2015). The Gut-Brain Axis: The Missing Link in Depression. Clinical Psychopharmacology and Neuroscience, 13(3), 239–244. http://doi.org/10.9758/cpn.2015.13.3.239

Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, Wolcott RD, Youn E, Summanen PH, Granpeesheh D, Dixon D, et al. Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe. 2010;16:444–453.

Finegold S.M., Molitoris D., Song Y., Liu C., Vaisanen M.L., Bolte E., Mcteague M., Sandler R., Wexler H., Marlowe E.M., Collins M.D., Lawson P.A., Summanen P., Baysallar M., Tomzynski T.J., Read E., Johnson E., Rolfe R., Nasir P., Shah H., Haake D.A., Manning P., Kaul A. Gastrointestinal microflora studies in late-onset autism. Clin. Infect. Dis. 2002;35:S6–S16.

Frohlich EE, Farzi A, Mayerhofer R, Reichmann F, Jacan A, Wagner B, et al. Cognitive impairment by antibiotic-induced gut dysbiosis: analysis of gut microbiota-brain communication. Brain Behav Immun. 2016. doi:10.1016/j.bbi.2016.02.020.

Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, Austin DW. Molecularcharacterisation of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Res. 2012;5:419–427.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. Proceedings of the National Academy of Sciences of the United States of America, 108(7), 3047–3052. http://doi.org/10.1073/pnas.1010529108.

Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, Mazmanian SK. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell. 2013;155(7):1451–63. doi: 10.1016/j.cell.2013.11.024.

Kang DW, Park JG, Ilhan ZE, Wallstrom G, Labaer J, Adams JB, Krajmalnik-Brown R. Reduced Incidence of and Other Fermenters in Intestinal Microflora of Autistic Children. PLoS One. 2013;8:e68322.

Kohane IS, McMurry A, Weber G, MacFadden D, Rappaport L, Kunkel L, Bickel J, Wattanasin N, Spence S, Murphy S, et al. The co-morbidity burden of children and young adults with autism spectrum disorders. PLoS One. 2012;7:e33224.

Liang S., Wang T., Hu X., Luo J., Li W., Wu X., et al. (2015) Administration of Lactobacillus helveticus ns8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. Neuroscience 310: 561–577.

Liu W.-H., Chuang H.-L., Huang Y.-T., et al. Alteration of behavior and monoamine levels attributable to Lactobacillus plantarum PS128 in germ-free mice. Behavioural Brain Research. 2016;298:202–209. doi: 10.1016/j.bbr.2015.10.046.

Liu X., Cao S., Zhang X. Modulation of gut microbiota–brain axis by probiotics, prebiotics, and diet. J Agric Food Chem. 2015;63:7885–7895.

Markota M, Sin J, Pantazopoulos H, Jonilionis R, Berretta S. Reduced Dopamine Transporter expression in the amygdala of subjects diagnosed with schizophrenia. Schizophrenia Bulletin. 2014;40(5):984-991. doi:10.1093/schbul/sbu084.

Maurer MH, Canis M, Kuschinsky W, Duelli R: Correlation between local monocarboxylate transporter 1 (MCT1) and glucose transporter 1 (GLUT1) densities in the adult rat brain. Neurosci Lett 2004, 355:105–108.

Nozawa, K., Kawabata-Shoda, E., Doihara, H., Kojima, R., Okada, H., Mochizuki, S., Ito, H. (2009). TRPA1 regulates gastrointestinal motility through serotonin release from enterochromaffin cells. Proceedings of the National Academy of Sciences of the United States of America, 106(9), 3408–3413. http://doi.org/10.1073/pnas.0805323106

Shaw W. Increased urinary excretion of a 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA), an abnormal phenylalanine metabolite of Clostridia spp. in the gastrointestinal tract, in urine samples from patients with autism and schizophrenia. Nutr Neurosci. 2010 Jun;13(3):135-43.

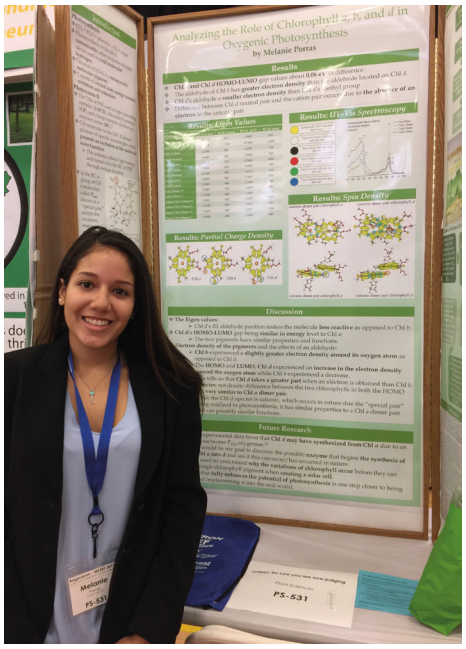
Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, Taylor R, Cain DP: Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism. Neuropharmacology 2008, 54:901.

Thomas RH, Meeking MM, Mepham JR, Tichenoff L, Possmayer F, Liu S, MacFabe DF: The enteric bacterial metabolite propionic acid alters brain and plasma phospholipid molecular species: further development of a rodent model of autism spectrum disorders. J Neuroinflammation. 2012, 9: 153-10.1186/1742-2094-9 153.

Yano JM, Yu K, Donaldson GP, et al. : Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell. 2015;161(2):264–76. 10.1016/j.cell.2015.02.047.

Zhang LS, Davies SS. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. Genome Med. 2016. doi: 10.1186/s13073-016-0296-x.

Analyzing the Role of Chlorophyll a, b, and d in Oxygenic Photosynthesis, by Melanie Porras (STS Scholar, Paper excerpt) (Senior)



Abstract

Oxygenic photosynthesis, the process of splitting water to synthesize carbon dioxide into glucose and produce oxygen, is effortlessly used by an abundance of plants every day. However, scientists have not yet been able to understand exactly how this process works. Many cofactors participate in the process of photosynthesis, one of them being chlorophyll. Though higher plant species use chlorophyll a and chlorophyll b in oxygenic photosynthesis, discoveries of a new chlorophyll have been made: chlorophyll d. This study analyzes different chlorophyll pigments in the intent to discover why plants evolved to use certain pigments. The methods used during this experiment was the use of density functional theory (DFT) and analyzing spectral properties from absorption spectra taken. Through further comprehension of the various cofactors in photosynthesis we are continuing to develop our understanding of how to replicate a photosynthetic process. The results of this study determined that not only is chlorophyll's spectral properties and function affected by its chemical composition but by also by the position of each element.

Introduction

A persistent problem humankind faces is the quest for a renewable energy source. In previous centuries, civilizations have relied heavily on fossil fuels but the burning of fossil fuels is detrimental to both local and global environments resulting in researchers searching for new and renewable energy sources. Nuclear, wind, and solar energy harvesting technologies have been developed but each has its own drawback. One of the most promising is solar energy because the sun produces significant amounts of energy providing a promising renewable energy source for the future. Modern solar technologies have low absorbencies, are too expensive, or have yet to be commercialized, meaning they can produce electricity suitable for now but cannot sustain the needs of billions of people. Scientists are now looking at natures solar cells for inspiration taking note of the structural and mechanistic properties that allow cyanobacteria and plants flourish, even in low light conditions.

Plants have created a highly efficient solar cell, with efficiencies ~ 3x greater than commercialized solar cells. This process, known as photosynthesis, uses sunlight to oxidize water yielding molecular oxygen and primarily carbohydrates. Moreover, this is accomplished with abundant, non-toxic, biodegradable components. Photosynthesis captures the sunlight reaching the surface and provides much of the energy and all the oxygen the remaining creatures need to survive. Billions of years of evolution culminated into the success of photosynthesis leading scientists to try and recreate photosynthesis in the lab. Moreover, since photosynthesis in the lab is customizable, solar xidation of water can be used to yield molecular hydrogen, or conduct CO2 reduction to simple organic compounds like formaldehyde or even methanol.

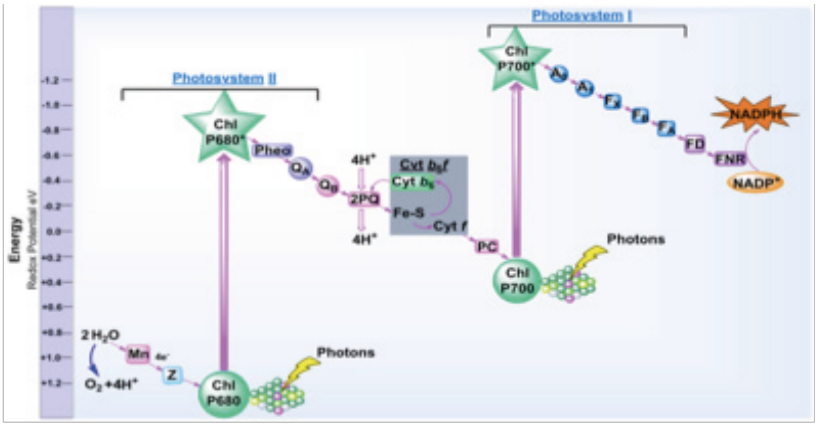


Figure 2: Shows Z-scheme of photosynthesis in higher order plants. Mn = Mn_4Ca-oxo cluster; Z = tyrosine; Chl P_{680} = PSII chlorophyll "special pair;" P_{680}* = excited P_{680}; Pheo = Pheophytin a; Q_A and Q_B = plastoquinones; Cyt b_6f = cytochrome b_6f; Cyt f = cytochrome f; Fe-S = iron sulfur cluster; Chl P_{700} = PSI chlorophyll "special pair;" P_{700}* = excited P_{700}; A_0 = chlorophyll a; FD = ferredoxin; FNR = ferredoxin NADP oxidoreductase.[1]

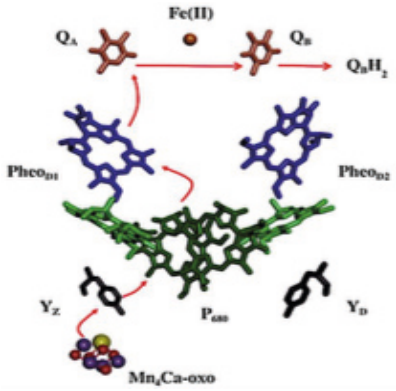


Figure 1: The electron transfer pathway in PSII. P_{680} is shown in dark green; pheophytins in purple; tyrosine (Yz and Y_D) in black; plastoquinones (Q_A and Q_B) in brown; and Mn_4Ca oxo cluster in red, purple, and yellow. This diagram was constructed from the 1.9 Å crystal structure of PSII from *Thermococcus Vulcanus* PDB ID 3WU2. [3]

tron transfer, making photosynthesis operate.[1] Chl is a magnesium chlorin which is capable of excited state energy transfer, oxidation and reduction, all of which enables photosynthetic reaction centers, or photosystems (PS) to conduct the oxidation of water releasing four protons and four electrons. These protons create a potential gradient which produces the ubiquitous energy molecule of life, ATP, while the electrons are used to store energy in the form of carbohydrates. The entire process of photosynthesis requires numerous protein complexes which are both cytoplasmic and transmembrane and for purposes of replication, photosystem II (PSII) is of primary focus because it conducts water oxidation.

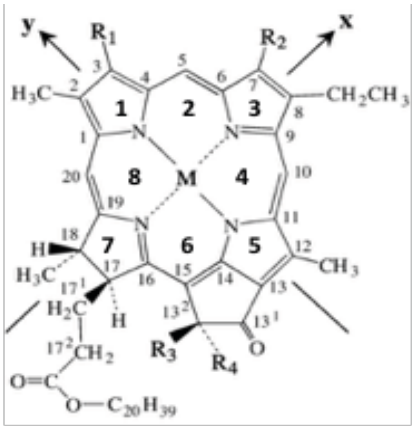
The Chls of PSII are packed into proteins and utilized as antenna in the light harvesting complex (LHCII), and chlorophyll proteins (CP) as well as and charge transfer cofactors in reaction center (RC).[2] Photosystem II (PSII) is located in the thylakoid membrane of chloroplast organelles which contains both LHC II and CPs to maximize light absorption. Initially, Chl molecules in the LHC II absorb photons of specific wavelengths which promote an excitation of the molecular wave function. Depending on the organism, the LHC II can contain various

Chls like Chl a, Chl b, or Chl d, as well as other pigments like carotenoids, to maximize photosynthetic capacity of PSII.1 This antenna collects light energy and funnels it to the RC of

PSII through exciton transfer. In the RC, a group of Chl a molecules, called P_{680}, known as a "special pair," accepts the excitation energy creating the excited state, P_{680}* (Figure 1). [3,4] The number included in the name of a "special pair" describes the certain wavelength it absorbs.

P_{680}* subsequently transfers an electron to, PheoD1, denoted by the red arrow in Figure 1. This transfer results in the charge separated state, $P_{680}^{\bullet+}$ $Pheo^{\bullet-}$ which is further stabilized by electron transferred from $Pheo^{\bullet-}$ to QA, the primary quinone acceptor. The electron is further transferred from $Qa^{\bullet-}$ to QB, the secondary plastoquinone and terminal electron acceptor creating the highly separated electron hole pair $P_{680}^{\bullet+}$ $QB^{\bullet-}$.[3] The highly oxidizing $P_{680}^{\bullet+}$ must be reduced in order to prevent damage to the protein complex. This reduction occurs via the redox active tyrosine residue, YZ, which undergoes proton-coupled electron transfer with a neighboring histidine residue. By losing a proton and an electron concurrently, the activation barrier is reduced and the intermediate is stabilized, allowing YZ to be reduced by the oxygen evolving complex (OEC), specifically a Mn_4Ca oxo cluster. The OEC undergoes successive oxidations storing four oxidizing equivalents and as two water molecules are oxidized; yielding 4 protons, 4 electrons, and molecular oxygen. [5] Here the OEC reaches its fully reduced state (S_0), resulting in a recovered state for the complex.

After the initial electron transfers in PSII, many more electron transfers are performed with other membrane bound protein complexes, like cytochrome b6f, and photosystem I (PSI) all the while facilitating proton transfer from the stroma to the lumen creating a proton gradient generating ATP. [1] Similar to PSII, the composition of the PSI complex has an antenna complex, light harvesting complex I (LHC I) and a RC which contains a group of chlorophylls referred to as P_{700} (Figure 2). P_{700} like P_{680}, has a special set of chlorophyll a molecules, however it consists of Chl a and Chl a', the 132-epimer of Chl a (Figure 3). A more distinctive characteristic of PSI compared to PSII is that the terminal electron acceptor is a redox active Fe_4S_4 cluster. [1,6] Energy absorption and electron transfer in PSI commences at LHC I. Through similar exciton transfer, energy is funneled to P_{700} creating the excited state P_{700}* (Figure 2). The special pair transfers an electron to the primary electron acceptor A0, a chlorophyll molecule creating the charge separated pair $P_{700}^{\bullet+}$ $A_0^{\bullet-}$. The charge separated pair is stabilized by further electron transfer to A_1, a second chlorophyll molecule, and subsequently transferred through FX, FB, and FA, a series of Fe_4S_4 clusters. $P_{700}^{\bullet+}$ is then re-reduced by plastocyanin, a reductant produced by cyt b6f, which converts electrons from PSII to plas-



	M	R_1	R_2	R_3	R_4
Chl *a*	Mg	$CH=CH_2$	CH_3	H	$COOCH_3$
Chl *a'*	Mg	$CH=CH_2$	CH_3	$COOCH_3$	H
Chl *b*	Mg	$CH=CH_2$	CHO	H	$COOCH_3$
Chl *d*	Mg	CHO	CH_3	H	$COOCH_3$
Chl *d'*	Mg	CHO	CH_3	$COOCH_3$	H

Figure 3: Shows the structural differences of Chl *a*, Chl *a'*, Chl *b*, Chl *d*, Chl *d'*.[7] Rings were also numbered.

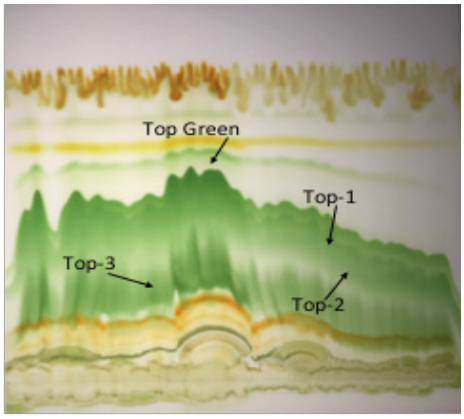


Figure 4: Shows the different colored bands after TLC was used. Middle green bands are chlorophyll.

tocyanin while also contributing the proton gradient, subsequently helping drive the synthesis of ATP.

The electrons generated from water splitting are transferred from PSII and continue throughout the electron transport chain and PSI to finally reduce ferredoxin and the ferredoxin-$NADP^+$ reductase, a flavoprotein; resulting in the production of NADPH. Once ATP and NADPH have been acquired the plant can start the chemical process of converting carbon dioxide into carbohydrates.

In recent years it has been discovered that Chl d exist as a dominant chlorophyll in the red algae, Acaryochloris marina.[7] While most oxygenic photosynthetic systems have mainly Chl a and low amounts of other accessory chlorophylls, A. marina consists of primarily Chl d and roughly 5% of Chl a.[8] In this unique cyanobacteria, Chl d not only serves as a light harvester of in LHCs but also as the main reaction center, believed to be an similar set of special Chls, P_{740}, in PSI.[7] Contrary to higher plants and other cyanobacteria which contain a Chl a/a' heterodimer in PSI, P_{740} in A. marina contains a Chl d/d' heterodimer.[6,7] However, the primary electron acceptor is still a chlorophyll a; similar to PSI in higher order plants and cyanobacteria. Although much is known about PSI of A. marina, the composition of the special pair in PSII is still a controversy. The possibilities consist of a Chl d dimer, 8 a Chl a dimer, 9 or a Chl a/d heterodimer. [10]

Statement of Purpose

In the present study, we analyze the structural differences of chlorophyll pigments, specifically Chl a, Chl b, and Chl d, to better understand how their chemical substitution changes, their spectral, and electrical properties elucidate as to why phototrophic organisms evolved to utilize different pigments. The structures of these three pigments are similar in their modifications yet vary in their spectral and chemical properties. All species are chlorins, which are [17-18] dihydro porphyrins. Chl a has a vinyl group at R_1 on the macrocycle and a methyl group at R_2 (Figure 3). The vinyl and methyl groups acts as weak electron donors while the vinyl group expands the π-system along the y-direction. Chl b is identical to Chl a except in the replacement of the R_2 methyl group with an aldehyde. This effect is important in that a weak electron donor has been replaced with a strong electron withdrawer as well as π-system alteration along the x-direction. Chl d varies in structure compared to Chl a in that the vinyl group in R_1 has been replaced with an aldehyde group, which retains π-system conjugation, but replaces an electron donor with an electron withdrawer. Although the two vary in structure their function is very similar. An explanation for this could be answered by the theory that states that Chl d may have been synthesized from Chl a due to a presence of a certain enzyme, specifically cytochrome P450 oxygenate.11 Here in this study we use absorbance spectroscopy and density functional theory (DFT) to analyze the effects of substitution on the partial charge distributions and spin density distributions of the highest occupied molecular orbitals (HOMO) and lowest unoccupied molecular orbitals (LUMO). We also support experimental findings with theoretical methods to understand substitution and structural effects on the spectral and electrochemical properties of

Chl a, Chl b, and Chl d, to better understand their properties and to elucidate why nature has evolved to use these pigments.

(...)

Results

Quantum Mechanical Calculations

VASP DFT computations were completed and analyzed to construct partial charge and spin density maps for each molecules HOMO and oxidized state.

Partial Charge Density

The electron density maps show an approximation of charge distributions for molecular orbital. The calculated HOMO charge densities for chlorophyll a, b, and d are depicted in Figure 5. The density appears constant with density existing on N1-N3 but not on N4, the saturated ring. Charge density is also observed across the Cα-Cβ bonds of rings 1-3 with some on the bridging carbons. Chl d was the only Chl to yield charge density on the nitrogen of ring 4. This density is due to the extensive conjugation experienced by planar π-systems. In addition, density can be seen hyper-conjugating with

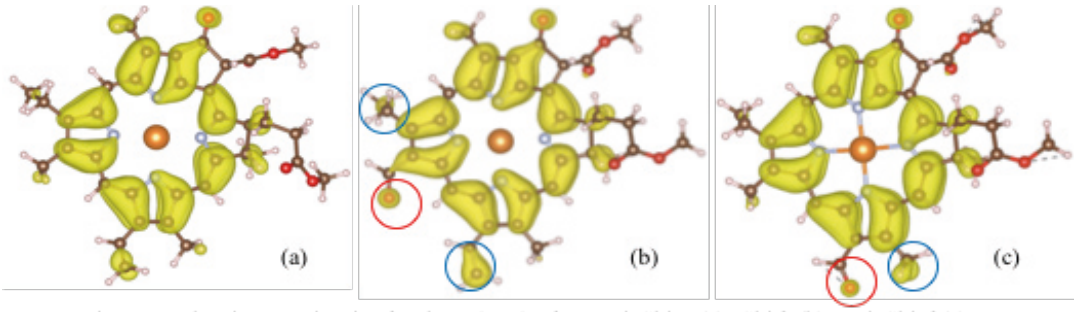


Figure 5: The electron density for the HOMO of neutral Chl *a* (a), Chl *b* (b), and Chl *d* (c). The aldehydes are circled in red and the affected substituents are circled in blue. [17]

the substituents of C2, C7, C8, C12, C17 and C18. This is partially why these groups are the electron donors. Conjugation is also observed to extend to the π-system of substituents were differences in electron densities are readily observed. According to our calculations, the aldehyde of Chl b has greater electron density than the aldehyde located on Chl d. This change in electron density at the aldehyde also affects nearby substituents as the C2 methyl group has a greater electron density compared to Chl a. Similarly, the aldehyde located in Chl b yielded an increase in electron density of nearby substituents.

The special set of Chls observed in organisms are referred to as such because of their cooperative work. Therefore, it was expected that the calculated HOMO of the Chl dimers had electron density distributed over both pigments. Each Chl of the dimer has charge density matching the distribution of the monomeric models shown in Figure 5. The main differences observed is that the Chl a dimer has a much more uniform charge distribution over the two compared to the Chl d dimer, both antarafacial and suprafacial (See Figure 6). In addition, the Chl d dimer exhibited charge density on the ligating histidine nitrogen to the Chl with more density. Calculations of the LUMO of each dimer pair were yielded, for both the antarafacial and superfacial, the Chl d dimer pair exhibited a bit of electron density on the Chl with less density in the HOMO.

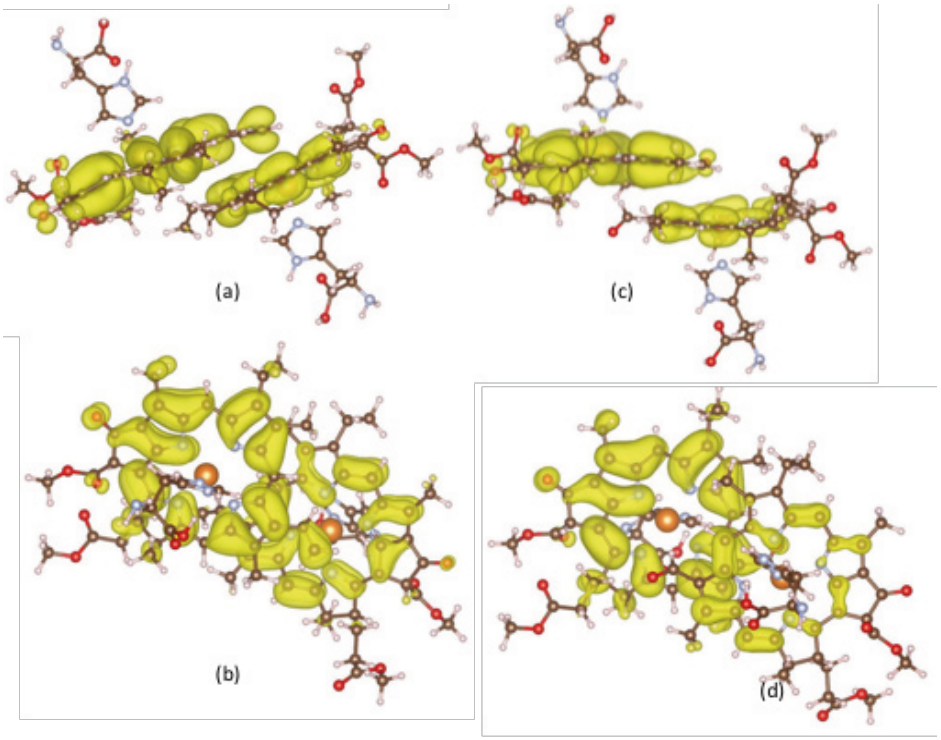


Figure 6: Shows the Highest Occupied Molecular Orbital (HOMO) for a neutral Chl *a* dimer pair side view (a) and top view (b). On left shows the HOMO for a neutral Chl *d* dimer pair (antarafacial) side view (c) and top view (d). [17]

Spin Density

The spin density distributions portra where spin can be found in the spin up (yellow) or spin down (cyan) state. In these calculations, the spin referred to is that of an unpaired electron. Since spin density represents the net spin of an entire molecular wave function, and in the case of monomeric Chl can be an anion or cation, anionic and cationic models of Chl a, b, and d were calculated. In nature when Chlz, accessory chlorophyll in the PSII RC, gets photo-oxidized it becomes a cation, positively charged, thus in this study the cationic species was more greatly studied. When analyzing the oxygen atom in an aldehyde of cationic Chl b and Chl d, the unpaired electrons surrounding Chl b had a net spin of spin up, meanwhile Chl d had a net spin of spin down (See Figure 7). However, when looking at the anions of each chlorophyll it was the opposite, Chl b had a net spin of spin down and Chl d had a net spin of spin up. When analyzing the presence of an aldehyde and its effect on the HOMO to LUMO of a neutral and cationic chlorophyll, and the effect of a HOMO-1 to HOMO for the anion, differences were also observed. Overall chlorophyll b experienced a decrease in electron density around the oxygen atom in an aldehyde, while chlorophyll d experienced an increase.

The spin density values for the Chl a and Chl d dimer pairs were also calculated and recorded. Overall the Chl d dimer pair (antarafacial) has a greater spin density than the Chl a pair (See Figure 8). Specifically, the Chl d dimer pair had a great

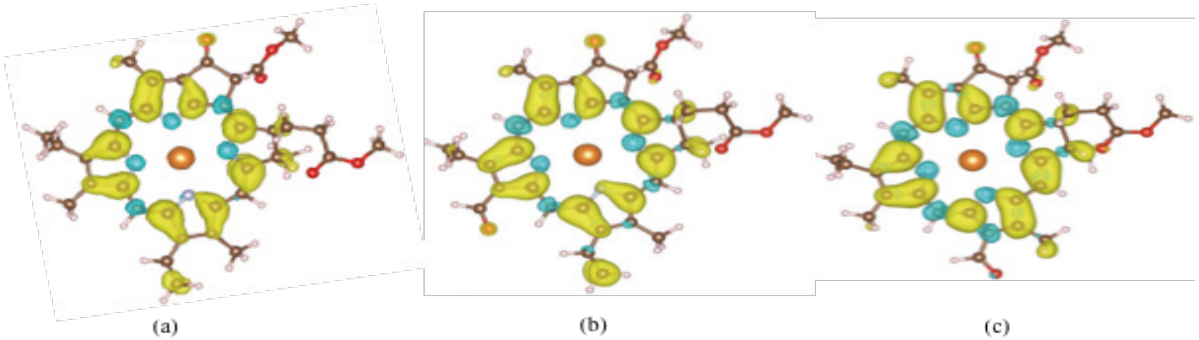


Figure 7: Shows the spin density of cations Chl *a* (a), Chl *b* (b), and Chl *d* (c). [17]

Molecule	H->L+1 (eV)	H->L+1 (nm)	H->L (eV)	H->L (nm)
Chl a Neutral	2.1744	570	1.5067	823
Chl a Anion	0.7112	1,743	0.7494	1,654
Chl a Cation	2.2188	559	1.6237	764
Chl b Neutral	1.8603	666	1.5832	783
Chl b Anion	1.3551	915	0.2482	4995
Chl b Cation	1.9684	630	1.6552	749
Chl d Neutral	2.1302	582	1.4514	854
Chl d Anion	1.4039	883	0.7601	1631
Chl d Cation	2.1981	564	1.5584	796
Chl a Dimer N	1.3257	935	1.2633	981
Chl a Dimer C	1.3990	886	1.3491	919
Antara Chl d Dimer N	1.3161	942	1.2232	1014
Antara Chl d Dimer C	1.3728	903	1.3437	923
Supra Chl d Dimer N	1.3412	924	1.2107	1024
Supra Chl d Dimer C	1.3924	890	1.3064	949

Table 1: Shows the HOMO-LUMO+1 gap and the HOMO-LUMO gap for all molecules in electronvolts (eV) and nanometers (nm).

spin down density (seen in blue) than Chl a. Interestingly noted, Chl d's aldehyde (circled in red) experience a smaller electron density than Chl a's methyl group (circled in blue). However, when direction is taken into consideration, Chl d's aldehyde was slightly shifted towards the other chlorophyll. When looking at the cation species of the Chl d dimer pairs, the LUMO of the Chl d dimer pairs contained similar electron density on both chlorophylls. This difference between the Chl d neutral pair and the cation pair occurs due to the absence of an electron in the cationic pair.

Eigenvalues

The eigenvalues of each molecule were calculated as well; showing the occupancy and energy of spin up and spin down electrons in each molecular orbital (MO). From here the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO) can be identified, and the HOMO-LUMO gap, measured in electronvolts (eV), can be calculated (See Table 1). Molecules that contains small HOMO-LUMO gaps are typically more reactive than molecules with larger gaps. 19 Gaps between MOs can be used to identify molecules because in a UV-Vis absorption spectra the Soret band represents the HOMO to LUMO+1 gap and the Q band shows the HOMO to LUMO gap. With the single chlorophylls the order of energy levels for both the HOMO to LUMO+1 gap and the HOMO to LUMO gap went as follows from greatest to smallest: the neutral chlorophyll, the chlorophyll anion, and then the chlorophyll cation. Since small HOMO-LUMO gaps are normally more reactive, it can be concluded that overall a chlorophyll anion is more reactive than the neutral and cationic species.

More specifically it was the Chl b anion that proved to be the most reactive, with an HOMOLUMO gap of 0.2482 eV, out of all molecules tested. Overall the Chl a and Chl d HOMOLUMO gap values were close in range, about 0.06 eV at most in difference. In terms of the chlorophyll dimer pairs in all HOMO-LUMO+1 and HOMO-LUMO gap (for all dimer pair species) there was roughly a .05 eV difference calculated. UV-Vis Spectroscopy of Pigment Extraction A UV-Vis spectroscopy of the all pigments and each band was done. Graph 1 shows the absorbance spectra of the pigment extract and each green band.

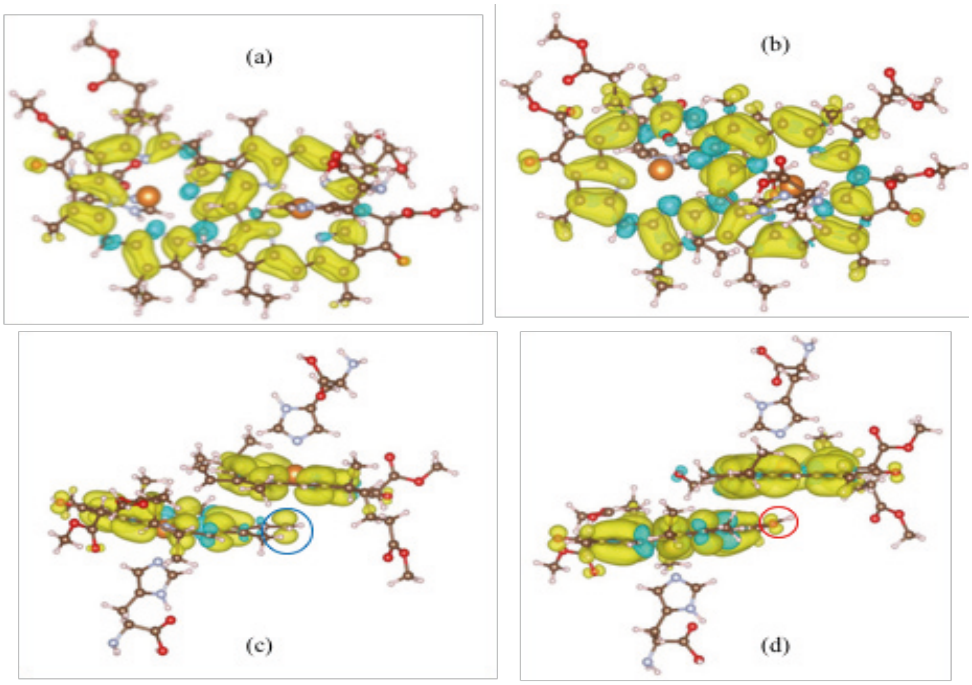


Figure 8: Shows the spin density of cationic dimer pair chlorophyll a top view (a) and bottom view (b); and cationic dimer pair chlorophyll d (antara) top view (c) and bottom view (d). Chl *d*'s aldehyde is circled in red and Chl *a*'s methyl group is circled in blue. [17]

From here the absorption spectra of the green bands was compared to the absorption spectra of known chlorophyll dissolved in methanol from Kobayashi's paper "Physicochemical properties of chlorophylls in oxygenic photosynthesis – succession of co-factors from anoxygenic to oxygenic photosynthesis." The green bands were then identified as chlorophyll a for they had a similar absorption peaks to ones measured: 432.5 nm and 665.8 nm.6

Discussion/Conclusion

In this experiment we analyzed the structural differences of Chl a, Chl b, and Chl d, to help us understand why certain organisms evolved to use different pigments. Though the structure of these pigments contain similar variations, the effect of elements in certain positions is critical.

While the vinyl and methyl groups acts as weak electron donors the aldehyde acts as a strong electron withdrawer. Although the aldehyde in Chl b alters the π-system along the x-direction, Chl d's aldehyde retains the π-system conjugation.

From quantum mechanical calculation, specifically calculating the Eigen values, it can be concluded Chl d's R1 aldehyde position makes the molecule less reactive as opposed to Chl b. Chl d's HOMO-LUMO gap being similar in energy level to Chl a tells us that these two pigments have similar properties and functions. A molecule's reactivity is crucial in photosynthesis as it cannot be easily disposed to reacting with everything its environment but rather to certain elements. When looking at the electron density of the pigments and the effects of an aldehyde it was concluded that Chl b experienced a slightly greater electron density around its oxygen atom as opposed to Chl d. Yet when looking at the effect of aldehydes on the HOMO and LUMO of these pigments, Chl d experienced an increase in the electron density around the oxygen atom while Chl b experienced a decrease. This tells us that Chl d takes a greater part when an electron is obtained than Chl b. When looking at the HOMO and LUMO of a neutral Chl d dimer pair (antara) certain characteristics were seen. In this case one of the chlorophylls of the pair experienced a greater electron density over the other in both the HOMO and LUMO. But, the cationic species didn't experience such a drastic difference between the two chlorophylls in both the HOMO and LUMO; very similar to the results found with the Chl a dimer pair. This tells us that while the Chl d species is cationic, which occurs in nature due the "special pair" being oxidized in photosynthesis, it has similar properties to a Chl a dimer pair and can possibly similar functions. Giving us a further information on the possibilities of the "special pair" in Acaryochloris marina's PSII. Further investigation would need to be gone to officially determine if A. marina PSI's Chl d/d' heterodimer evolved from a Chl a dimer pair.

Since Chl d is difficult to obtain it would be part of my future studies to try and synthesize Chl d from the Chl a we obtained. Past research has not yet defined an exact answer to replicate this process, thus it would be my goal to discover the possible enzyme that commences the synthesis of Chl a into d and see if this can occur/has occurred in nature.

Once we discover why photo-oxidizing organisms evolved the way they did (for survival or due to location; just to name a few) then a system that fully enhances the potential of photosynthesis is one step closer to being created. Scientists need to understand why the variations of chlorophyll occur before they can choose a single chlorophyll pigment when creating a solar cell. Once this obstacle has been overcome then commence the process of replicating artificial photosynthesis on a solar panel and implementing it into the real world.

Mentor:
Dr. K. V. Lakshmi, Ph.D.

ASSOCIATE PROFESSOR Chemistry and Chemical Biology.

SCIENTIFIC DIRECTOR of the Baruch '60 Center for Biochemical Solar Energy Research

Rensselaer Polytechnic Institute.

We are grateful to Dr. Lakshmi and Dr. G. Theophall for their dedication and help.

References

1. Senge, M.O.; Ryan, A.A.; Letchford, K.A.; MacGowan, S.A.; Mielke, T.. Chlorophylls,

Symmetry, Chirality, and Photosynthesis. Symmetry 2014, 6, 781–843.

2. McConnell, I.; Li, G.; Brudvig, G. W. Energy Conversion in Natural and Artificial Photosynthesis. Chemistry and Biology 2010, 17, 434–447.

3. Lakshmi, K.V.; Coates, C.S.; Smith, S., Chatterjee; R. The Radical Intermediates of Photosystem II. The Biophysics of Photosynthesis, Biophysics for the Life Sciences 2014, 299–320.

4. Allakhverdiev, S. I.; Kreslavski, V. D.; Zharmukhamedov, S. K.; Voloshin, R. A.;

Korol'kova, D. V.; Tomo, T; Shen, J.-R. Chlorophylls d and f and their role in primary photosynthetic processes of cyanobacteria. Biochemistry (Moscow) 2016, 81–201.

5. Velthuys, B.; Kok, B. Photosynthetic oxygen evolution from hydrogen peroxide. Bioenergetics 1978, 221–221.

6. Kobayashi, M.; Akutsu, S.; Fujinuma, D.; Furukawa, H.; Komatsu, H.; Hotota, Y.; Kato, Y.; Kuroiwa, Y.; Watanabe, T.; Ohnishi-Kameyama, M.; Ono, H.; Ohkubo, S.; Miyashita, H.

Physicochemical properties of chlorophylls in oxygenic photosynthesis – succession of cofactors from anoxygenic to oxygenic photosynthesis. In Zvy Dubinsky, Photosynthesis 2013, 47–90.

7. Kobayashi, M.; Ohashi, S.; Iwamoto, K.; Shiraiwa, Y.; Kato, Y.; Watanabe, T. Redox potential of chlorophyll d in vitro. Elsevier 2007, 596–602

8. Nieuwenburg, P,; Clarke, R.J.; Cai, Z.L.; Chen, M.; Larkum, A.W.D.; Cabral, N.M.; Ghiggino, K.P.; Reimers, J.R. Examination of the photophysical processes of chlorophyll d leading to a clarification of proposed uphill energy transfer processes in cells of Acaryochloris marina, Photochem. Photobiol. 2003, 77, 628–637.

9. M. Mimuro; S. Akimoto; T. Gotoh; M. Yokono; M. Akiyama; T. Tsuchiya; H. Miyashita; M. Kobayashi; I. Yamazaki, Identification of the primary electron donor in PS II of the Chl d dominated cyanobacterium Acaryo-chloris marina,

FEBS Lett. 2004, 556, 95–98.

10. Kobayashi, M.; Watanabe, S.; Gotoh, T,; Koizumi, H.; Itoh, Y.; Akiyama, M.; Shiraiwa, Y.;

Tsuchiya, T.; Miyashita, H.; Mimuro, M.; Yamashita, T.; Watanabe, T. Minor but key chlorophylls in photosystem II, Photosynth. Res. 2005, 84, 201–207.

11. Allakhverdiev, S.I.; Kreslavski, V.D.; Zharmukhamedov, S.K.; Voloshin, R.A.; Korol'kova, D.V.; Tomo, T.; and Shen, J.R. Chlorophylls d and f and Their Role in Primary Photosynthetic Processes of Cyanobacteria. Biochemistry 2016, 201–2012

12. Hoffmann, R.; Woodward, R. B. J. Cycloaddition Reactions. Am. Chem. Soc. 1965, 87(9), 2046-2048

13. Kresse, G.; Furthmüller, J.; Efficient Iterative Schemes for Ab-Initio Total-Energy Calculations Using a Plane-Wave Basis Set. Physical Review B. 1996, 54, 169–186.

14. Perdew, J.P.; Burke, K.; Ernzerhof, M.; Generalized Gradient Approximation Made Simple.Physical Review Letters 1996, 77, 3865–3868.

15. Umea, Y.; Kawakami, K,; Shen, J.R.; & Kaiya, N. Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. Nature 2011, 473, 55–60

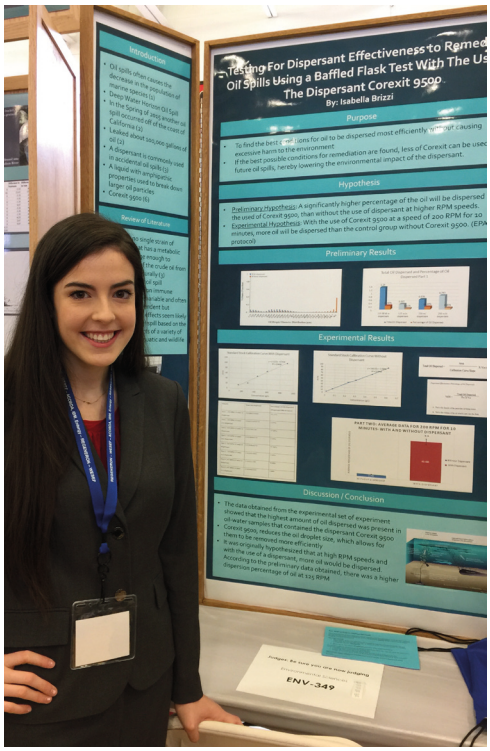
16. Hanwell, M.D.; Curtis, D.E.; Lonie, D.C.; Vandermeersch, T; Zurek, E; Hutchison, G.R. "Avogadro: An Advanced Semantic Chemical Editor, Visualization, and Analysis Platform." Journal of Cheminformatics 2012, 4–17.

17. Plata, J. Leaf chlorophyll content. Instrumentation for studying vegetation canopies for remote sensing in optical and thermal infrared regions, 1990, 5, 207–213.

18. Kresse, G.; Furthmüller, J.; Efficient Iterative Schemes for Ab-Initio Total-Energy Calculations Using a Plane-Wave Basis Set. Physical Review B. 1996, 54, 169–186.

19. Zhang, G.; Musgrave, C.B. Comparison of DFT Methods for Molecular Orbital Eigenvalue Calculations. J. Phys. Chem. A 2007, 111, 1554–1561

Testing For Dispersant Effectiveness to Remediate Oil Spills Using a Baffled Flask Test With The Use of The Dispersant Corexit 9500 by Isabella Brizzi (STS paper excerpt) (Senior)



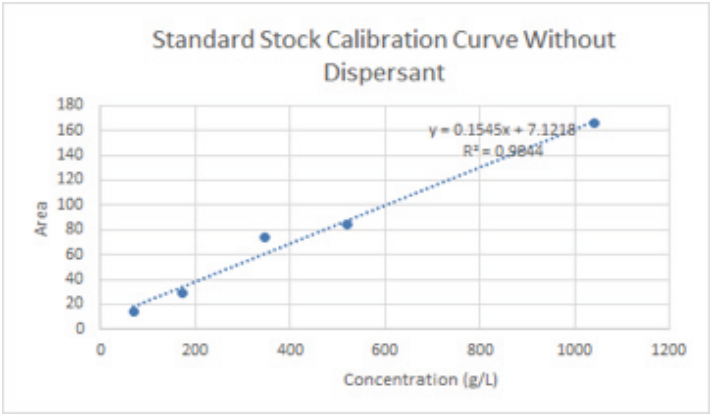
Abstract:

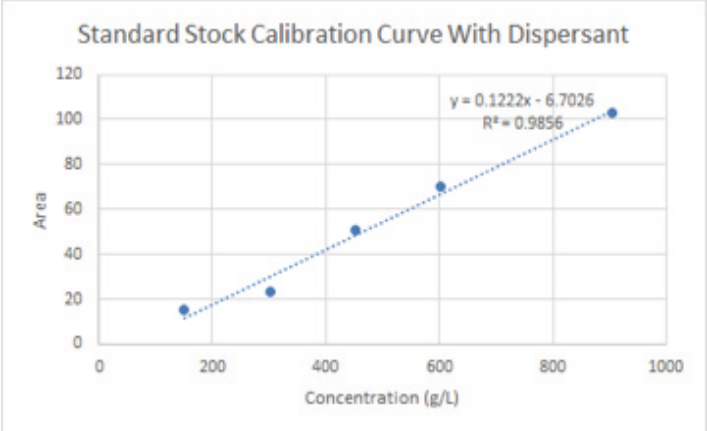
Corexit9500, a dispersant for accidental oceanic oil spills, was studied to determine the most efficient parameters for its use while reducing environmental impact. In our initial study, a baffled flask test performed at 125 RPM and 250 RPM, with and without dispersant for two hours to mimic wave patterns (7). Liquid- liquid extracts were then measured in a UV spectrophotometer to determine the percent oil dispersed. Our initial hypothesis (at higher RPM speeds and in the presence of a dispersant, more oil would be dispersed) was not supported in the preliminary experiments: More oil was dispersed at lower RPM speeds with the presence of a dispersant. Based on these results, we modified the procedure; and all groups were tested with and without the use of a dispersant, at 200 RPM for 10 minutes, allowing for less oil sticking to the baffled flask. This new data set showed that more oil was dispersed in the presence of Corexit9500, even though some of the data showed that oil was still lost to the walls of the baffled flask. More studies have to be conducted to confirm that this effect is consistent and replicable, thus the need for more trials to be completed.

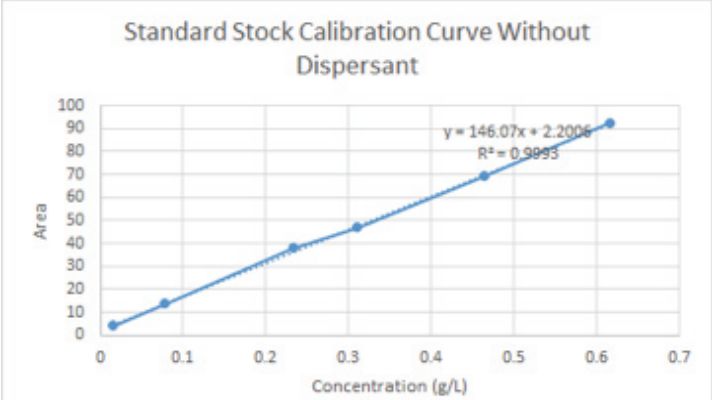
Introduction:

Oils spills have been a prevalent issue in society since The Deepwater Oil Spill in April of 2010. Oil spills like the Deepwater Horizon Oil spill, often are responsible for causing a population of species to become drastically affected, resulting in death within the population. In consequence, such disasters may cause a significant decrease in a species population (7). The Deepwater Horizon Oil Spill caused thousands of gallons of oil to be leaked into the Gulf of Mexico, which affected many aquatic species of wildlife (1). An example of

Figure 1: (a) Standard Stock Calibration Curve data with the use of a dispersant (Part One). Graph displays Area vs the Concentration of oil in grams per liter. (b) Standard Stock Calibration Curve data without the use of a dispersant (Part One). Graph displays Area vs the Concentration of the oil in grams per liter. The dashed line represents the line of best fit.







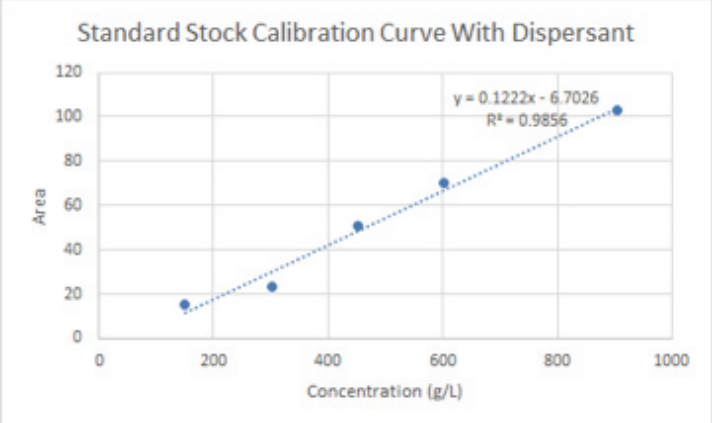


Figure 5: (a) Standard Stock Calibration Curve data with the use of a dispersant (Part Two Graph displays Area vs the Concentration of oil in grams per liter. (b) Standard Stock Calibration Curve data without the use of a dispersant (Part Two). Graph displays Area vs the Concentration of the oil in grams per liter. The dashed line represents the line of best fit.

a similar situation would be the increase of deaths in the Dolphin species following Hurricane Sandy. Many of the dolphins who washed up on beaches after Hurricane Sandy were seen to have died of the Dolphin Morbillivirus, a virus belonging to the measles family (9). Recently in the spring of 2015, another oil spill occurred off the coast of California releasing an estimated 105,000 gallons of oil released, due to a pipeline rupture (2). In order to attempt to remediate the damage caused by these disasters, the use of a dispersant is needed. A dispersant is a liquid or gas that is commonly used to help clean up oil spills. Dispersants can break down larger particles of oil into smaller particles. This allows the particles to be able to break down further and be consumed by microorganisms (3).Exposure to deadly toxins or chemicals can weaken an organism's immune system and make that organism more susceptible to various diseases (1). Although many of the effects of dispersants are still unknown, modern day dispersants are less toxic than those previously used (6). If the correct dispersant is used at the found optimal condition, it can be expected that the effect on both the environment and the wildlife can be less severe.

Goals and Hypothesis

Part One:

The purpose of Part One of the experiment would be to find the most effective condition for a dispersant to work most efficiently without causing any unnecessary harm to the environment. To do so, we would test for the best possible conditions for the dispersant, Corexit 9500, working with a baffled flask at various RPMs. At RPM speeds with the use of a dispersant, more oil would be dispersed, facilitating its removal. The different RPM speed would be used to stimulate different wave patterns, creating an ocean like situation. It is expected to find a significantly higher percentage of the oil dispersed with the use of this dispersant in the best possible conditions. Without the use of a dispersant, there would not be a percentage of oil dispersed at the RPMs tested.

Part Two:

Based on the results of Part One, our hypothesis was modified: at 200 RPM with the use of a dispersant, more oil would be dispersed as compared to without the use of dispersant.The purpose of Part Two of this experiment would be to follow the EPA's protocol to collect data on dispersant effectiveness at 200 RPM for 10 minutes, to see how well the dispersant, Corexit 9500 works more efficiently without causing any unnecessary harm to the environment. It was originally hypothesized that at higher RPM speeds, with the use of a dispersant, more oil would be dispersed. (...)

Samples	Total Oil Dispersed	Percentage of Oil Dispersed (Dispersant Effectiveness)
125 RPM w/ dispersant	1.29	21.6%
125 RPM w/o dispersant	.307	5.11 %
250 RPM w/ dispersant	.576	15.2 %
250 RPM w/o dispersant	.797	8.85 %

Figure 2: Table displays data from Total Oil dispersed and Percentage of Oil dispersed equations at 125 RPM and 250 RPM with and without the use of a dispersant, at 2 hours (where the most conclusive data was seen).

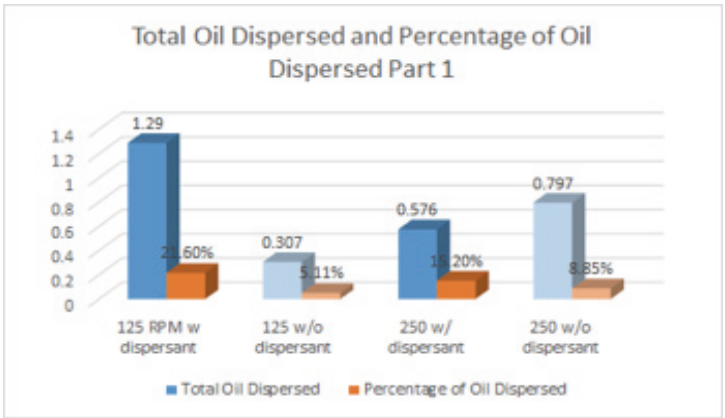
Results:

Below are the equations necessary to determine the total amount of oil dispersed.

Area: Based on absorbance at 340,370, and 400 nm from the UV spectrometer measurements

(((Abs 340+Abs 370) X 30) /2) + (((Abs 370 + Abs 400) X 30) / 2)

Figure 3: Bar graph compares data from Total Oil Dispersed and Percentage of Oil Dispersed at 125 RPM and 250 RPM with and without the use of a dispersant, Corexit 9500. Bar graph shows data from 2 hours.



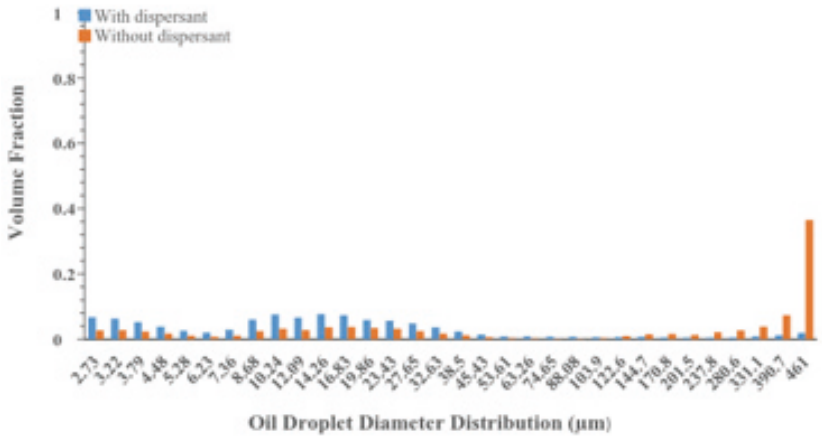


Figure 4: LISSTX100 graph displays data from Part one, comparing the Volume Fraction to the Oil Droplet Diameter Distribution in micrometers.

The Area formula was used to find the amount of total oil that would be dispersed as well as its percentage (5).

Total Oil Dispersed (calibration curve slopes, obtained from Figure 1 and Figure 5):

V DCM is the volume of the Dichloromethane.

V tw is the total volume of seawater that is used in the baffled flask.

V ew is the total volume of seawater that is extracted from the flask.

Dispersant Effectiveness (Percentage of Oil Dispersed)

Poil is the density of the particular oil being tested.

Voil is the volume of the oil which is put into the flask-

$$\%OD = \frac{\text{Total Oil Dispersed}}{P_{oil} \times V_{oil}}$$

Part 1:

Calibration Curves

The standard curves (Figure 1 and Figure 5) obtained for Part One and Part Two, are necessary for determining the Total Oil Dispersed in each Baffled Flask. The slope of the line of best fit from each of the standard stock curves is used with their corresponding sample to determine the Total Oil Dispersed in each Baffled Flask. For example, a baffled flask test at 200 RPM without dispersant, would use the slope from the Part 2 Standard Stock Calibration curve without dispersant (Figure 5(b)). The standard stock Calibration Curves have much less room for error because they are not conducted within a baffled flask, and thus there is no oil to be lost to the walls of the flask during its shaking period.

The table results from Figure 2 are from Part One of the experiment. The results were taken from a two hour sample. Part One's results showed that more oil was dispersed at lower RPM speeds which was unexpected. It was originally hypothesized that at higher RPM speeds and with the use of a dispersant, more oil would be dispersed. My data showed that at lower RPM speeds with the use of a dispersant, more oil was dispersed. The reason for this is because at high RPM speeds, such as 250 RPM, a majority of the oil was lost to the walls of the baffled flask.

$$\text{Total Oil Dispersed} = \frac{\text{Area}}{\text{Calibration Curve Slope}} \times V_{DCM} \times \frac{V_{tw}}{V_{ew}}$$

The oil- water samples from Part One were placed into a LISSTX100 machine to test for the volume fraction as well as the oil droplet diameter distribution in micrometers. As seen in Figure 4, the oil droplet size in micrometers was much larger in particles that were not in contact with the dispersant, Corexit 9500. A larger droplet size, also correlated to larger volume fraction. In Part Two of the experiment, the procedures were altered to follow the EPA's protocol in order to receive the most accuracy. The data showed that within the presence of Corexit 9500, the oil droplets size was successfully reduced, allowing for the oil droplets to be remediated more efficiently. Even though the results from Part One of the experiment were different from what was originally hypothesized, the data from the LISSTX100 machine shows that no matter the condition, Corexit successfully reduces the oil droplets size.

Part 2:

Calibration Curves

The table from Figure 6, shows four different tests from Part Two, with and without dispersant at 200 RPM for 10 minutes each. For each of these tests, the raw data from the total oil dispersed, as well as the percentage of oil dispersed (dispersant effectiveness) is shown on the table. This data was calculated using the slope from the calibration curve slopes in Figure 5. The raw data from the table shows that with the use of the dispersant, the percentage of oil dispersed was significantly higher than without the use of a dispersant.

As seen in Figure 7, the raw data from the table in Figure 6 was taken and put into a statistical data analysis program. The chart in Figure 7 shows the averages of the percentage of oil dispersed with dispersant and the percentage of oil dispersed without dispersant. The average without dispersant is 2.765 %, which is considerably lower than the average with dispersant which is 40.485 %. This data shows that in the presence of Corexit 9500, more oil is dispersed than without the addition of a dispersant. This result is highly significant, as assessed with a t-test and confirms the preliminary results as seen in part 1, the use of dispersant is critical in increasing the percentage of oil dispersed.

Discussion:

In both Part One and Part Two of the experiment it was seen that oil-water samples with the dispersant, Corexit 9500, had a higher percentage of oil dispersed than compared to the samples that

Samples	Total Oil Dispersed	Percentage of Oil Dispersed (Dispersant Effectiveness)
Test 1: 200 RPM 10 mins w/ dispersant	0.02889332004	33.21%
Test 1: 200 RPM 10 mins w/o dispersant	0.002211953173	2.54%
Test 2: 200 RPM 10 mins w/ dispersant	0.032792136	37.69%
Test 2: 200 RPM 10 mins w/o dispersant	0.0028096118	3.23%
Test 3: 200 RPM 10 mins w/ dispersant	0.0399840479	45.96%
Test 3: 200 RPM 10 mins w/o dispersant	0.0026124461	3.00%
Test 4: 200 RPM 10 mins w/ dispersant	0.039221336	45.08%
Test 4: 200 RPM 10 mins w/o dispersant	0.0019963031	2.29%

Figure 6: Table displays Part Two's raw data from 4 identical tests, tested at 200 RPM, with and without the use of a dispersant. Each sample was tested at a time of 10 minutes, following the EPA's protocol for accuracy

Mentor:
Dr. Zhong Pan, Ph.D.

Post Doctoral Research Associate.

Center for Natural Resources Development and Protection, New Jersey Institute of Technology

We are very grateful to Dr. Pan for his dedication and help.

were tested without dispersant. Corexit 9500, a dispersant with amphipathic properties, has been used to clean oil spills in environmental disasters (11). This data shows that Corexit 9500, effectively disperses the oil droplets allowing them to become smaller particles. As the oil particles become smaller, they can then be consumed by microorganisms, such as bacteria (3). The loss of oil in Part One of the experiment due to the walls of the baffled flask made for a larger margin of error and confused the results. The original variation, observed in the Part One experiments, in the future could be fixed by testing it again in future studies with the addition of Sylon CT. When the walls of the baffled flask are lined with Sylon CT, a majority of the oil is stopped from sticking the walls of the baffled flask (11). It may be beneficial to test Sylon CT in both Part One and Part Two of the experiment to see how it affects the percentage of oil dispersed by Corexit 9500 in each sample. If higher percentages of oil dispersed are seen, and Sylon CT is deemed environmentally safe, it may be used in addition to Corexit 9500 in future oil spill remediation. Part One's preliminary data of the experiment was conducted at a time of two hours at RPM speeds of 125 and 250 RPM. It was seen that at higher RPM speeds, a significant amount of the oil was lost to the baffled flask itself. In part two of the experiment, each sample was shaken for a time for 10 minutes at 200 RPM and it was seen that there was much less oil residue left the walls of the baffled flask, giving more conclusive data. More studies need to be completed to confirm this data to test and optimize other conditions for its use. In the future it would be interesting to consider the use of Corexit 9500 in other bodies of water like rivers, which have fewer waves, thus correlating to very low RPM speeds.

Conclusion:

Oil Spills have become an increasingly popular issue within today's modern society. As the environment is constantly changing due to global warming and the rising of sea levels, the world has become more suscepti-

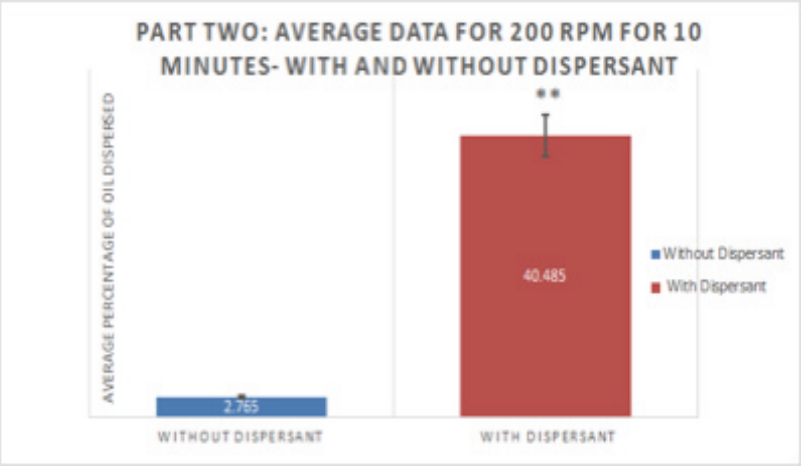


*Figure 7: Chart displays the statistical averages for Part Two's data at 200 RPM for 10 minutes, with and without dispersant. The average for the percentage of oil dispersed without the dispersant Corexit 9500 is 2.765 %, while the average for the percentage of oil dispersed with the dispersant Corexit 9500 is 40.485 %. The averages for both the with and without dispersant within Figure 7 have error bars which represent the standard error of the mean. n=4, ** indicate p<0.001 in unpaired t-test*

ble to natural disasters such as oil spills. Oil dispersants are likely to be used very often, as more of these disasters may occur. They do have detrimental effects on the immune system of marine mammals and thus it is imperative to optimize the methodology for their use, such as lowering concentrations based on wave patterns, and finding the most efficient protocol to disperse the oil (1). The research that I have conducted over the course of two summers is more important than ever now, and it seeks to find a condition for remediation that is both successful and efficient, allowing for the least damage to be brought to the environment as possible.

References

Barron, Mace G. "Ecological impacts of the deepwater horizon oil spill: implications for immunotoxicity." Toxicologic pathology 40.2 (2012): 315-320.

"California oil spill: Up to 105,000 gallons lost - CNN.com." 2015. 24 Nov. 2015 <http://www.cnn.com/2015/05/20/us/california-oil-spill/>

Venosa, A. D., & Zhu, X. (2003). Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. Spill Science & Technology Bulletin, 8(2), 163-178.

Venosa, Albert D, Dennis W King, and George A Sorial. "The baffled flask test for dispersant effectiveness: a round robin evaluation of reproducibility and repeatability." Spill Science & Technology Bulletin 7.5 (2002): 299-308

Venosa, Albert D, and Edith L Holder. "Determining the dispersibility of South Louisiana crude oil by eight oil dispersant products listed on the NCP Product Schedule." Marine pollution bulletin 66.1 (2013): 73-77.

US Environmental Protection Agency. (2014, December 31). COREXIT® EC9500A.Retrieved November 02, 2016, from https://www.epa.gov/emergency-response/corexitr-ec9500a

Zhao, L., Wang, B., Armenante, P. M., Conmy, R., & Boufadel, M. C. (2016). Characterization of Turbulent Properties in the EPA Baffled Flask for Dispersion Effectiveness Testing. J. Environ. Eng. Journal of Environmental Engineering, 142(1), 04015044. doi:10.1061/(asce)ee.1943-7870.0001000

Somerville, G. A., & Proctor, R. A. (2013, January 16). Cultivation conditions and the diffusion of oxygen into culture media: The rationale for the flask-to-medium ratio in microbiology. BMC Microbiology BMC Microbiol, 13(1), 9. doi:10.1186/1471-2180-13-9

Venn-Watson, S., Garrison, L., Litz, J., Fougeres, E., Mase, B., Rappucci, G., Rowles, T. (2015). Demographic Clusters Identified within the Northern Gulf of Mexico Common Bottlenose Dolphin (Tursiops truncates) Unusual Mortality Event: January 2010 - June 2013. PLOS ONE PLoS ONE, 10(2). doi:10.1371/journal.pone.0117248

Kaku, V. J., Boufadel, M. C., Venosa, A. D., & Weaver, J. (2006). Flow Dynamics in Eccentrically Rotating Flasks Used for Dispersant Effectiveness Testing. Environmental Fluid Mechanics, 6(4), 385-406. doi:10.1007/s10652-006-0003-3

Campo, P., Venosa, A. D., & Suidan, M. T. (2013). Biodegradability of Corexit 9500 and Dispersed South Louisiana Crude Oil at 5 and 25 °C. Environmental Science & Technology Environ. Sci. Technol., 47(4), 1960-1967. doi:10.1021/es303881h

Detecting Antecedents of Boredom in Students Using Intelligent Tutoring Systems by Matthew Gofman (STS paper excerpt) (Senior)

Abstract

Boredom is rampant throughout students participating in online education and it is imperative to be able to predict which students will become bored before they reach that point. We use data from the intelligent mathematics tutoring system ASSISTments. Within this study, we attempt to create a model that best predicts boredom in students utilizing intelligent tutoring systems by using a computational detector of boredom as the label. In order to develop the model, we had to engineer seventeen additional features through distillation of the data set given to us by ASSISTments. Our goal is for the model to be able to accurately predict the onset of boredom within students and to reveal specific student actions which act as boredom antecedents. Understanding cues which precede boredom would enable researchers to better understand the causes of boredom and allow educators to more effectively diminish the adverse impacts boredom has on students by intervening when the antecedents are detected. While our model did not reveal to be very predictive of boredom, out model did point out specific student actions which can, with a level of statistical significance higher than others, be used as antecedents of boredom, providing a significant framework for future research

Introduction

Boredom is considered to be an epidemic of contemporary society as one of the most widespread affects (Spacks, 1995). However, boredom receives significantly less research than alternate emotions (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). It is often assumed that this lack of research is a result of boredom being an unassuming and often taciturn affect, as opposed to other emotions such as apprehension or anger (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). While seemingly discreet, the impacts of boredom are just as detrimental (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). Boredom is a parlous emotion that has been shown to be detrimental to

the learning process (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010).

Boredom is associated with irregular conduct (Wasson, 1981), absences (Wasson, 1981), and dropping out of school (Bearden, Spencer, & Moracco, 1989).

Despite its profound impact on the educational process, antecedents of boredom are seemingly neglected in research (Daschmann, Götz, & Stupnisky, 2014). Being able to discover antecedents is integral to being able to create boredom intervention programs in educational settings (Daschmann, Götz, & Stupnisky, 2014). It is only after educators understand antecedents of boredom that they can alter their teaching system to alleviate boredom within students (Daschmann, Götz, & Stupnisky, 2014).

Previous research projects have discovered personal factors as antecedents of boredom (Niculescua, Tempelaara, Dailey-Hebertb, Segersa, & Gijselaersa, 2015; Daschmann, Götz, & Stupnisky, 2014) and have shown that the variance in the individual problems is more predictive of boredom than the variance in the students (Hawkins, Heffernan, & Baker, 2013). One of the most accepted sources from which researchers have discovered antecedents of boredom in the past is from the personal perceptions of the students (Kunter & Baumert, 2006). According to this approach, students understand when they are bored and why they became bored (Daschmann, Götz, & Stupnisky, 2014). Research has also revealed the relationship between the student's recognition of boredom and the educator's perception of the antecedents (Daschmann, Götz, & Stupnisky, 2014).

Theoretical Antecedents of Boredom

Theoretical viewpoints on antecedents of boredom exist (Goetz & Frenzel, 2006). Pekrun's control-value theory is often used a theoretical model for predicting boredom (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). This model pronounces environmental and subjective causes. The subjective, or individual, antecedents are due to the student's perception over control over their specific situation and their assessment of worth (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). Boredom may occur if the student believes that they lack control or have too much of it (Pekrun, Frenzel, Goetz, & Perry 2007). The theory also describes that students may become bored if they believe that there is little value in what they are doing (Pekrun, Goetz, Daniels, Stupnisky, & Perry, 2010). The environment around the student, such as the teaching caliber, can also dictate the emotions of the students (Goetz, Frenzel, Stoeger & Hall, 2010). Another possible explanation is that the principle antecedent of boredom is monotony (Hill & Perkins, 1985). If a student believes that a situation is repetitive, the student is likely to become bored (Hill & Perkins, 1985). Hill and Perkins specify that the task, the situation, and the person themselves all influence boredom development (Hill & Perkins, 1985).

Purpose of Study

The purpose of this study is to develop a model that is able to detect student actions within intelligent tutoring systems that are predictive of boredom. To our knowledge, researchers have yet to attempt to discover boredom antecedents on the clip-level, such as specific student actions that could predict boredom. Our goal is for this model to be able to predict, with a level of statistical significance, which non-bored students will become bored in the future, using certain cues that act as boredom antecedents. Having knowledge of what precedes boredom would be invaluable to educators and developers of intelligent tutoring systems. Understanding certain actions that precede boredom would allow educators to intervene when such actions are detected, thus potentially preventing the onset of boredom. Developers of online tutoring platforms could program the platform to change the learning direction when the antecedents are detected. Thus, discovering antecedents of boredom would lead to a decrease of boredom within students in educational settings and lead to increase in learning.

Methodology

We used data from ASSISTments, an online intelligent mathematics tutoring system that is used weekly by around 40,000 middle school students (Hawkins, Heffernan, & Baker, 2013). ASSISTments evaluates the students as they are working, and sends the data to the teacher (Hawkins, Heffernan, & Baker, 2013). The students are also provided with support in answering the questions, receiving either hints, feedback, or scaffolding (Hawkins, Heffernan, & Baker, 2013). Hints come in various levels, proceeding from high-level hints to hints that provide the answer (Hawkins, Heffernan, & Baker, 2013). Feedback is provided if the student provides erroneous responses (Hawkins, Heffernan, & Baker, 2013). Scaffolding splits the problem into numerous smaller problems in order to assist the student in the process (Hawkins, Heffernan, & Baker, 2013). For this study, we used data that had formerly been gathered from ASSISTments. The data includes around 6,000,000

Dr. Ryan Baker, Ph.D.

ASSOCIATE PROFESSOR of Cognitive Studies in Education

Teachers College, Columbia University, NY

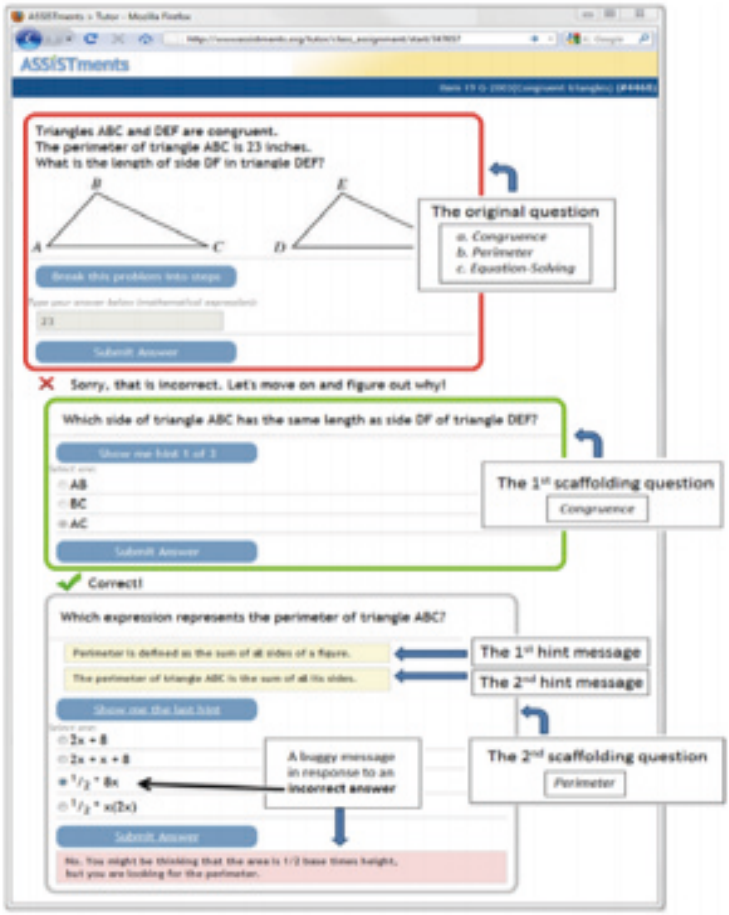


Figure 1: ASSISTments Example (Gowda, Baker, Pardos, & Heffernan, 2011

student actions gathered from 2012-2013. ASSISTments provides de-identified and publicly available data for researchers. Included in the data set is an affect detector, in addition to information regarding the start time of the problem, the end time of the problem, nature of the first attempt (correct or incorrect/hint), whether the student asked for all available hints, the total number of hints asked for, the total number of attempts on the problem, the time it took for the student to make their first action, among other variables (Table 1) (Pardos, Baker, San Pedro, Gowda, & Gowda, 2009).

Every action includes a real-value confidence that boredom was existent during that action as a number between 0 and 1, with numbers closer to 1 indicating a higher probability that boredom was present (Pardos, Baker, San Pedro, Gowda, & Gowda, 2009). This acted as the detector's projected likelihood that the student was bored when they made a specific action (Hawkins, Heffernan, & Baker, 2013). The detector was created by merging ASSISTments log files with thousands of boredom field observations (Hawkins, Heffernan, & Baker, 2013).

In order to determine the best predictive model of boredom, we had to perform additional data distillation on the ASSISTments data set. By creating additional features, we were able to create a more accurate and predictive model than by simply using the data given. Through modifying an existing software base (Goldstein), we engineered seventeen additional features that could be used to develop a more predictive model of boredom (Table 2).

Statistical Analysis and Results for Aggregated Features

Methodology

The only data utilized within the model were the actions that fell within the antecedent period of the detector's prediction of boredom. Our initial study attempted to develop a model containing features that aggregated all values within the problem into the average, max, min, and standard deviation of the values. For example, one column would contain the average of all the values within a specific feature during the problem, while another would contain the max, min, and standard deviation of the values within that feature. These aggregated features were developed for all of the variables that were included in the data set and those which we had developed.

In order to determine the most predictive model of boredom, we fit REPTree regression models to the data in order to predict the boredom detector confidence. REPTree utilizes regression tree logic, developing numerous trees in a multitude of iterations, ultimately revealing the most predictive tree (Ka-

Provided Data Utilized (Table 1)

Feature	Function
StartTime	The timestamp for when the problem began.
EndTime	The timestamp for when the problem concluded.
Original	Indicates whether the problem was a main problem or a scaffolding problem.
Correct	Determines whether the student was correct on the first attempt or whether they were either incorrect on the first attempt or asked for help.
BottomHint	Indicates if the student asked for all possible hints.
HintCount	Tallies the total number of hints the student used on the problem.
AttemptCount	Gives the number of times the student has attempted an answer on the problem.
MsFirstResponse	Provides the time it took for the student to make their first response, in milliseconds.
FirstAction	Determines whether the first action was an attempt or whether it was a hint request.
AverageConfidenceBored	The detector's projected likelihood, between 0 and 1, that the student was bored during that action.

Engineered Features (Table 2)

Feature	Function
TotalPastWrongCount	Tallies the total amount of times that the student provided an incorrect answer on the first attempt while attempting problems of the same skill in the past.
TotalPercentPastWrong	Provides the summation of the percent of times that the user has provided an incorrect answer on the first attempt while attempting problems of the same skill in the past.
Past8BottomOut	Tallies the number of problems within the last eight that ended using a bottom out hint, or the last hint in a sequence.
IsHelpRequest	Determines if the action was a first response, and if so, whether or not help was requested.
TotalTimeOnSkill	Determines the total time the student has spent on the skill.
TimeSinceSkill	Provides a value in seconds which reveals the amount of time it has been since the last time a problem of the same skill type had been seen by the student.
WorkingInSchool	Determines if the action was made during school hours, defined as between 7:00 a.m.–2:59 p.m., Monday-Friday.
TotalAttempted	Provides the total number of problems the student has attempted using the system. This is done to determine how experienced the student is in using the system.
TotalSkillOpportunities	Provides the total amount of unique problems that the student has attempted on the skill they are currently working on.
ResponseIsFillIn	Reveals if the response type is "fill in one" or algebra. In general, this translates to a more developed knowledge base for a middle school student.
ResponseIsChosen	Reveals if the response type is a "choose one" question in which the student has to pick the answer after being given multiple possible answers. In these questions, the student has a larger chance of picking the correct choice through guessing.
EndsWithScaffolding	Determines whether or not the problem concludes with scaffolding.
EndsWithAutoScaffolding	Determines whether or not the problem concludes with automatic scaffolding.
TimeTakenOnScaffolding	Provides the time taken on a scaffolding problem.
TotalSkillOpportunitiesScaffolding	Determines the amount of unique problems the student has seen on the skill they are currently on.
TotalFrSkillOpportunitiesByScaffolding	Calculates the result of dividing the number of scaffolding problems on the skill by the number of total problems on the skill.
IsHelpRequestScaffolding	Determines if the action is the first response to a problem, and if so, whether help was requested.

lmegh, 2015). To evaluate the models, we used Spearman's rho measures. Spearman's rho is a nonparametric correlation coefficient that measures to what extent two variables are related based on the monotonic relationship (Laerd). The closer the Spearman's rho value is to ±1, the stronger the relationship is (Weir).

Results

Our initial REPTree analysis with 10 parameters resulted in a Spearman's rho of 0.136. Values within the range of .00-.19 are considered to have a very weak monotonic relationship (Weir). While our initial model was a rather poor predictor of boredom, it did provide a framework for the development of a better model. While overall not very predictive, the regression tree revealed which features are the most predictive of boredom. "Hint" was the most predictive feature, followed by "TotalTimeOnSkill," "TotalSkillOpportunities," "ResponoseIsChosen," "Correct," and "TimeSinceSkill". "Hint" provides a value of 0 or 1, indicating whether or not a student asked for a hint during that action. "TotalTimeOnSkill" determines the total time the student has spent on the skill. "TotalSkillOpportunities" provides the total amount of unique problems that student has attempted on the skill they are currently working on. "ResponseIsChosen" reveals if the response type is a "choose one" question in which the student has to pick the answer after being given multiple possible answers. In these questions, the student has a larger chance of picking the correct choice through guessing. A value of 0 or 1 is given in "Correct," with a 1 indicating that the student was correct on the first attempt, and 0 indicating the student was either incorrect on the first attempt, or asked for help (Hawkins, Heffernan, & Baker, 2013). "TimeSinceSkill" provides a value in seconds which reveals the amount of time it has been since the last time a problem of the same skill type had been seen by the student.

Statistical Analysis and Results for 'n-back' Features

Methodology

Aggregating the values of the data for each feature did not result in a model that could act as a statistically significant predictor of boredom. In place of aggregated features, we engineered 'n-back' features, which created a more predictive model. 'N-back' features refer to features which take the summation of all values a certain number, or n, actions back. For example, a 10-back "TimeSinceSkill" feature, would take the summation of the previous 10 "TimeSinceSkill" values on that problem. We took the 5-back, 10-back, 15-back, and 20-back values for every variable we had.

Results

The 'n-back' feature REPTree analysis with 10 parameters resulted in a better spearman's rho of 0.241, indicating a stronger monotonic relationship. While this monotonic relationship is still 'weak' per se, the large increase in the spearman's rho value after performing additional feature engineering is promising. This indicates that aggregating data distorts it in a way that makes it less predictive.

The REPTree analysis revealed that in general, the '5-back' variables were the most predictive, revealing that student actions become more predictive of boredom the closer the student is to becoming bored. Using the 'n-back' features as opposed

to the aggregates also resulted in differing results, with "Past8BottomOut," "TotalSkillOpportunities," "TotalPercentPastWrong," "AttemptCount," "Correct," and "BottomHint" being the most predictive features. "TotalSkillOpportunities" and "Correct" were also near the top of the most predictive features in our previous study. "Past8BottomOut" tallies the number of the eight past problems that ended using a bottom out hint, or the last hint in a sequence. "TotalPercentPastWrong" provides the summation of the percent of times that the user has provided an incorrect answer on the first attempt while attempting problems of the same skill in the past. "AttemptCount" gives the number of times the student has attempted an answer on the problem (Pardos, Baker, San Pedro, Gowda, & Gowda, 2009). "BotomHint" gives a 0 or 1 value indicating whether the students asked for all of the hints or not (Pardos, Baker, San Pedro, Gowda, & Gowda, 2009).

Conclusion, Implications & Further Research

Additional feature engineering through the 'n-back' features led to significant improvements in the model, leading to .105 increase in the Spearman's rho value. While the Spearman's rho still indicates a rather weak monotonic relationship, our initial results are still significant. While the overall model may not be a strong predictor of model, the model did reveal which specific variables are predictive of boredom. Additionally, our research shows that actions become more predictive of boredom the closer the student is to becoming bored. These variables can, with a level of statistical significance higher than others, be used to predict which students that are not currently bored will become bored in the future. Educators can utilize such features to notice specific values that are significant within the variable, and intervene in order to possibly prevent the student from becoming bored in the future. Programmers of online tutoring systems can also code the platform to change the learning process when significant values are detected within the features that are considered antecedents of boredom. The goal of my future research is to engineer even more features, and perform more statistical analyses, in hope of developing a more predictive model of boredom. This research has shown that the engineering of more variables will lead to a more predictive model.

References

Bearden, L., Spencer, W., & Moracco, J. (1989). A study of high school dropouts. School Counselor, 37, 113–120.

Daschmann, E., Götz, T., & Stupnisky, R. (2013). Exploring the antecedents of boredom: do teachers know why students are bored? Teaching and Teacher Education, 39, 22-30.

Goetz, T., & Frenzel, A. C. (2006). Phänomenologie schulischer Langeweile [Phenomenology of boredom at school]. Zeitschrift für Entwicklungspsychologie und Pädagogische Psychologie, 38(4), 149-153.

Goetz, T., Frenzel, A. C., Stoeger, H., & Hall, N. C. (2010). Antecedents of everyday positive emotions: an experience sampling analysis. Motivation and Emotion, 34(1), 49-6.

Goldstein, A.B. FeatureDistiller2null [Computer Program].

Gowda, S., Baker, R.S.J.d., Pardos, Z., Heffernan, N. (2011). The Sum is Greater than the Parts: Ensembling Student Knowledge Models in ASSISTments. Proceedings of the KDD 2011 Workshop on KDD in Educational Data.

Hawkins, W., Heffernan, N., Baker, R.S.J.d. (2013) Which is more responsible for boredom in intelligent tutoring systems: students (trait) or problems (state)? Proceedings of the 5th biannual Conference on Affective Computing and Intelligent Interaction.

Hill, A. B., & Perkins, R. E. (1985). Towards a model of boredom. British Journal of Psychology, 76(2), 235-240.

Kalmegh, Sushilkumar. (2015). Analysis of WEKA Data Mining Algorithm REPTree, Simple Cart and RandomTree for Classification of Indian News. International Journal of Innovative Science, Engineering & Technology, 2(2).

Kunter, M., & Baumert, J. (2006). Who is the expert? Construct and criteria validity of student and teacher ratings of instruction. Learning Environments Research, 9(3), 231-251.

Laerd (2016). Spearman's Rank-Order Correlation. Spearman's Rank-Order Correlation. Laerd Statistics.

Niculescua, A., Tempelaara, D., Dailey-Hebertb, A., Segersa, M., Gijselaersa, W. (2015). Exploring the antecedents of learning-related emotions and their relations with achievement outcomes. Frontline Learning Research, 3 (1), 1-17.

Pardos, Z.A., Baker, R.S.J.d., San Pedro, M.O.C.Z., Gowda, S.M., Gowda, S.M. (2013) Affective states and state tests: Investigating how affect throughout the school year predicts end of year learning outcomes. Proceedings of the 3rd International Conference on Learning Analytics and Knowledge, 117-124.

Pekrun, R., Frenzel, A. C., Goetz, T., & Perry, R. P. (2007). The control-value theory of achievement emotions: an integrative approach to emotions in education. In P. A. Schutz, & R. Pekrun (Eds.), Emotion in education (pp. 13-36). San Diego, CA US: Elsevier Academic Press.

Pekrun, Reinhard; Goetz, Thomas; Daniels, Lia M.; Stupnisky, Robert H.; Perry, Raymond P.

(2010). Boredom in achievement settings: Exploring control–value antecedents and performance outcomes of a neglected emotion. Journal of Educational Psychology, Vol 102(3), 531-549.

Spacks, P. M. (1995). Boredom: The literary history of a state of mind. Chicago, IL: University of Chicago Press.

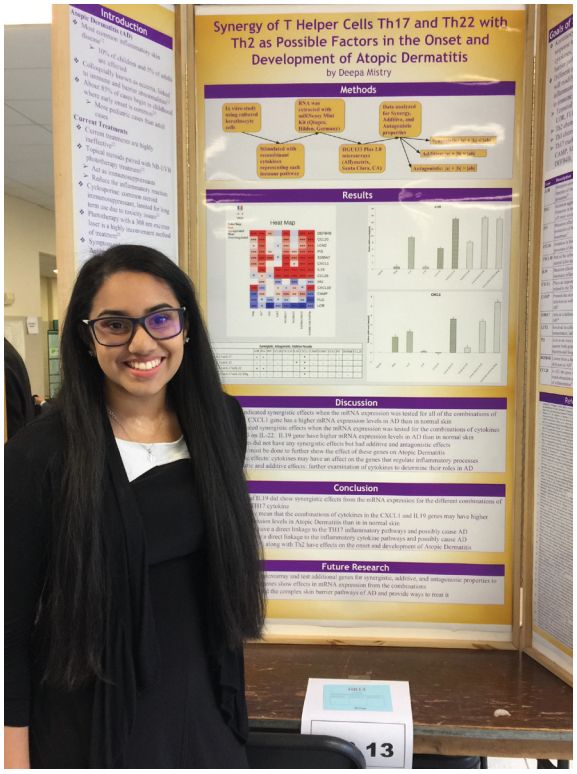
Wasson, A. S. (1981). Susceptibility to boredom and deviant behavior at school. Psychological Reports, 48, 901–902.

Weir, Iain. Spearman's Rank Correlation - Introduction. StatsTutor. StatsTutor.

Synergy of T Helper Cells T_H 22 and T_H 17 with T_H 2 as possible factors in the onset and development of Atopic Dermatitis by Deepa Mistry (STS paper excerpt) (Senior)

Abstract

Atopic dermatitis is an inflammatory skin disorder in which there are immune and barrier abnormalities in the skin. The root cause of Atopic Dermatitis is unknown and many studies have been completed to determine which genes and cytokines cause the disease. This experiment tests different genes for their expression after being exposed to different combinations of TH22, TH1, and TH17 cytokines. The results are preliminary but show that the genes CCL20, LCN2, PI3, DEFB4B, S100A7, CXCL1, IL19, CCL26, CXCL10, and CAMP were generally upregulated when targeted by different cytokines, and the genes INV, FLG, and LOR were downregulated. Of all of these genes, CXCL1 has the most synergistic properties with the combinations of cytokines presented. Though



some of the results were insignificant, the preliminary results can lead to a more in depth study on the root causes of Atopic Dermatitis.

Introduction:

Atopic Dermatitis is the most common inflammatory skin disease in which 10% of children and 5% of adults are affected [12]. It is an inflammatory skin disorder that is more colloquially known as eczema [12]. There are two important stages in atopic dermatitis that show the progression of the disease. The acute stage is the initial irritation of the skin where it looks bright red, "wet", and flat [12]. The chronic stage is the dull, red, dry, and thick aftermath from the acute stage [12]. This skin disorder is known to be closely linked to both immune and barrier abnormalities [12]. About 85% of cases begin in childhood where early onset is common and there are more pediatric cases of atopic dermatitis than adult cases [23].

Current treatments of atopic dermatitis are highly ineffective [23]. Topical steroids are sometimes paired with NB-UVB phototherapy treatment because both act as immunosuppressants which would reduce the inflammatory reaction associated with atopic dermatitis [23]. In addition, cyclosporine, a common immunosuppressant is also a popular treatment for this disease [23]. However, due to toxicity issues from the oral steroids and other immunosuppressants, there are limitations for long term use [23]. Phototherapy is given with a 308 nm excimer laser at a dermatologist's office and is usually administered 2-3 times a week with a 48 hour break in between treatments, making phototherapy a highly inconvenient method of treatment [23].

While treatments may be ineffective, there are ways to help manage atopic dermatitis. Some symptom reducing treatments include antihistamines and antibiotics [23]. With the highly inflamed skin, antihistamines work to reduce the irritation and reduce swelling [23]. The antibiotics are effective in cases where infections have developed on the irritated lesions [23].

Literature Review

The root of the immune pathways in atopic dermatitis lies in the T Helper cells which are cells that help to activate cytotoxic T cells to terminate abnormal cells or foreign bodies [12].

The current model for this pathway shows that the hallmark of T helper cells in atopic dermatitis is the T H 2 cell [12]. Keratinocytes work to form a barrier against foreign invaders of the skin. Inatopic dermatitis, the epidermal barrier formed by keratinocytes is disrupted, forming lesions of eczema [32].

Research has shown that treatments that block the TH2 axis can help atopic dermatitis patients because it reduces the severity of the disease [18]. Since it does not completely treat atopic dermatitis, it suggests however, there may be other cytokines involved [18].

There has also been research that shows TH22, TH1, and TH17 cytokines like IL-22, IL-17, are also involved in immune activation in atopic dermatitis [12]. The inflammatory mediator genes used are CCL26, CXCL10, IL-19, CXCL1, CAMP, S100A7, LCN2, PI3, DEFB4B, and CXCL20 in addition to the keratinocyte differentiation genes LOR, FLG, and INV. The LOR gene codes for Loricrin, a protein in the cornified cell envelope, a structure that serves as a protective barrier for the skin against the environment [21] . It is formed in fat cells underneath the

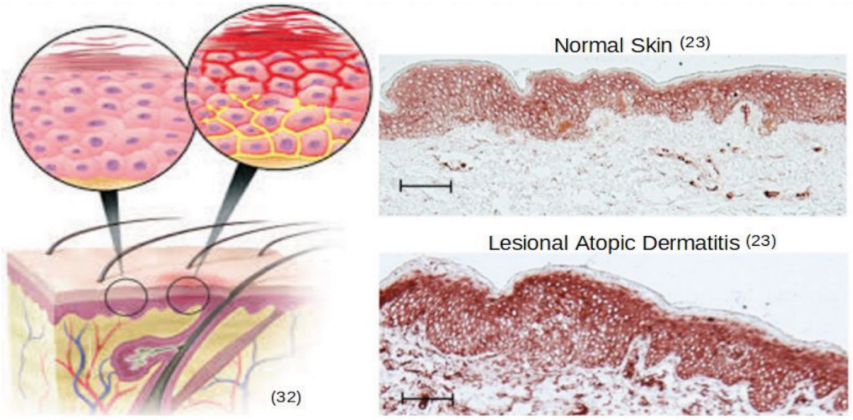


Figure 1: Normal Skin versus Atopic Dermatitis Lesions
The figure on the left shows that the epidermis is calm and no irritation in the left bubble and the epidermis on the left hand bubble is inflamed, red, and irritated. The Normal Skin figure on the upper right hand side shows the presence of cytokines in a normal skin sample. It shows that the epidermis is calm and without irritation or inflammation. The Lesion Atopic Dermatitis figure on the lower left hand side shows the presence of cytokines in a lesional atopic dermatitis skin sample. It shows that the epidermis is more inflamed, darker in color, and irritates.

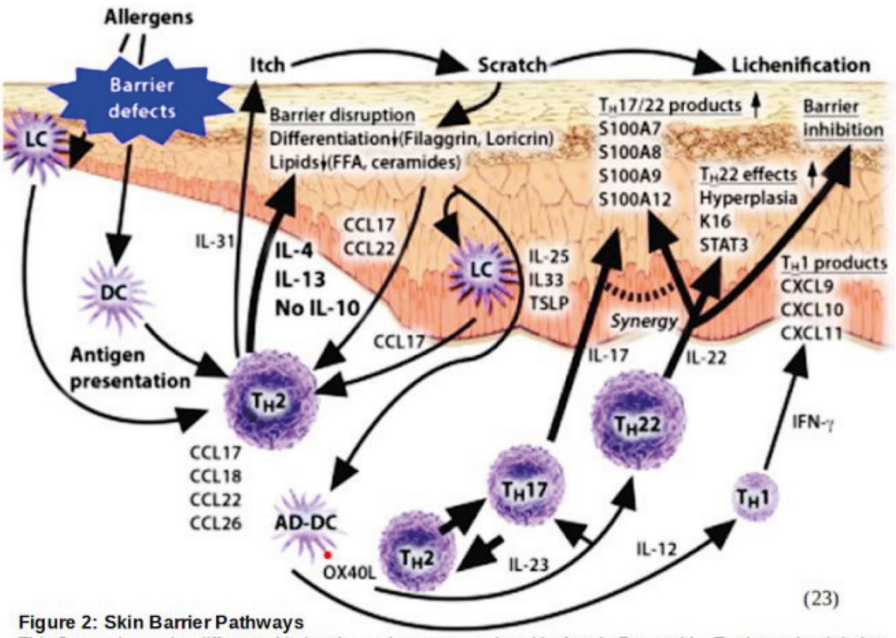


Figure 2: Skin Barrier Pathways
This figure shows the different skin barrier pathways associated in Atopic Dermatitis. Each purple globular structure represents a T Helper cell, which are cells that help to activate cytotoxic T cells to terminate abnormal cells or foreign bodies. The inflammatory mediator genes used are CCL26, CXCL10, IL-19, CXCL1, CAMP, S100A7, LCN2, PI3, DEFB4B, and CXCL20 in addition to the keratinocyte differentiation genes LOR, FLG, and INV, which are represented in the figure.

plasma membrane called terminally differentiated stratified squamous epithelium, or epidermal cells [21]. When a mutation occurs in LOR gene the protein malfunctions a barrier function of the skin [21]. The impacted barrier because of the defect on the LOR gene enables abnormal processes of the barrier and congenital disorders of the skin such as Atopic Dermatitis [21].

The FLG gene codes for Filaggrin, a protein that binds to the main structural component of hairs in epithelial cells [11] . A mutation in this gene aggravates the keratin intermediate filaments in the epidermis [11] . It is also more likely to make Atopic Dermatitis more severe in adulthood [11] .

The INV gene codes for Involcrin, a covalently bonded component in the keratinocyte crosslinked envelope [17]. It is found in the cytoplasm that by transglutaminase is crosslinked to the membrane proteins [17] . Involcrin is important to Atopic Dermatitis because of its involvement in the cornified cell envelope in stratified squamous epithelia [17]. These epithelial cells are flattened and layers on a basal membrane, protecting the tissue underneath [17] .

In Atopic Dermatitis, the cell's membranes are affected and there is poor protection of the tissue underneath the basal membrane [17]. The CCL26 gene is one of the two CC (Cys-Cys) genes [5]. They are involved in inflammatory processes and immunoregulatory processes

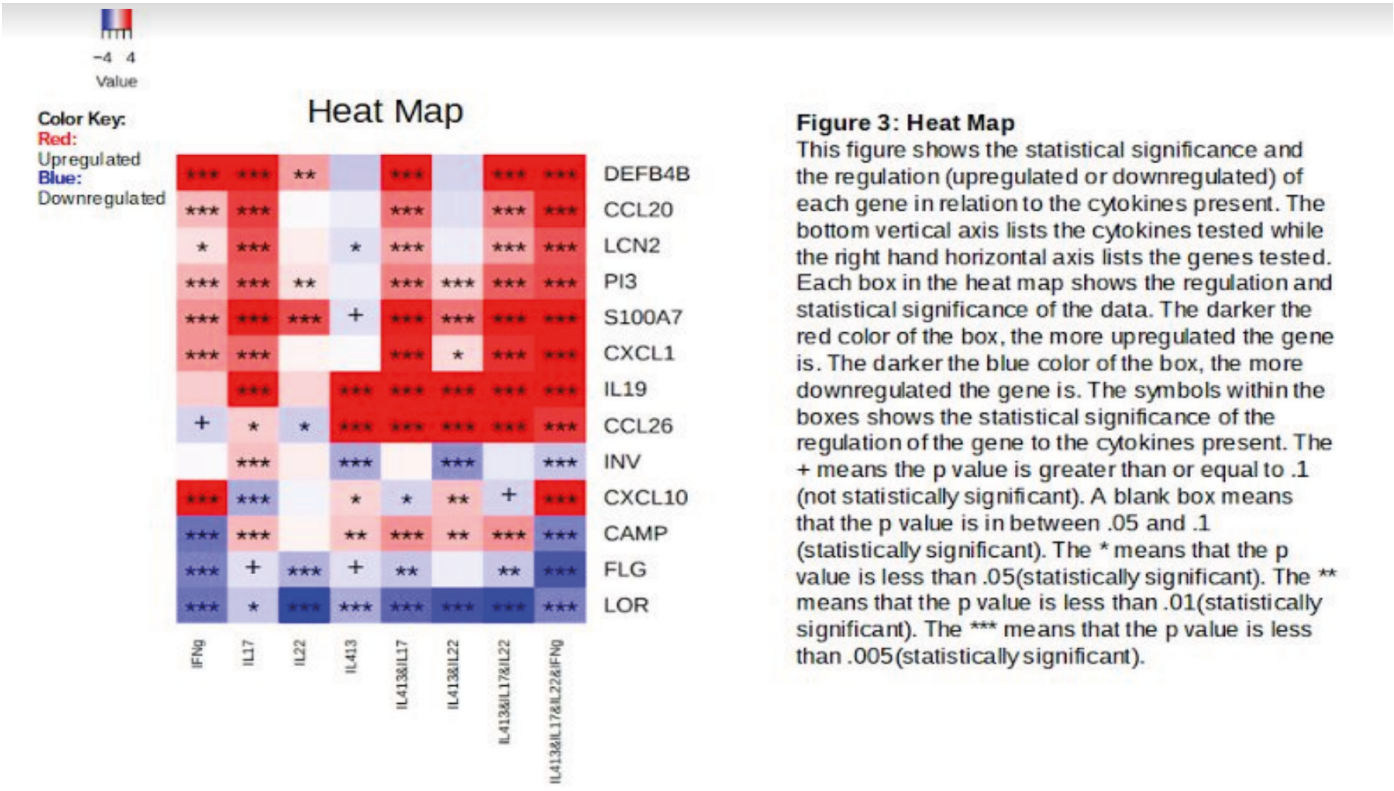


Figure 3: Heat Map
This figure shows the statistical significance and the regulation (upregulated or downregulated) of each gene in relation to the cytokines present. The bottom vertical axis lists the cytokines tested while the right hand horizontal axis lists the genes tested. Each box in the heat map shows the regulation and statistical significance of the data. The darker the red color of the box, the more upregulated the gene is. The darker the blue color of the box, the more downregulated the gene is. The symbols within the boxes shows the statistical significance of the regulation of the gene to the cytokines present. The + means the p value is greater than or equal to .1 (not statistically significant). A blank box means that the p value is in between .05 and .1 (statistically significant). The * means that the p value is less than .05(statistically significant). The ** means that the p value is less than .01(statistically significant). The *** means that the p value is less than .005(statistically significant).

which display chemotactic activity, the movement in response to chemical stimulus [5]. Since it is produced by inflammatory cells and expressed in endothelial cells, the mutation in this gene disrupts the inflammatory processes and therefore enabling Atopic Dermatitis to appear on the skin [5].

The CXCL10 gene is an antimicrobial gene [6]. It codes for a chemokine, a signaling protein, of the CXC subfamily and the receptor CXCR3 ligand [6]. This gene is part of the inflammatory cascade in Atopic Dermatitis and it also acts as a natural killed and T-cell migration. It is secreted by interferon gamma [6].

The IL19 gene is a protein coding gene that codes for a cytokine in the IL19 subfamily [16]. It is expressed in monocytes, large phagocytic white blood cells [16]. It codes for an inflammatory cytokine, maintaining inflammation in Atopic Dermatitis [16].

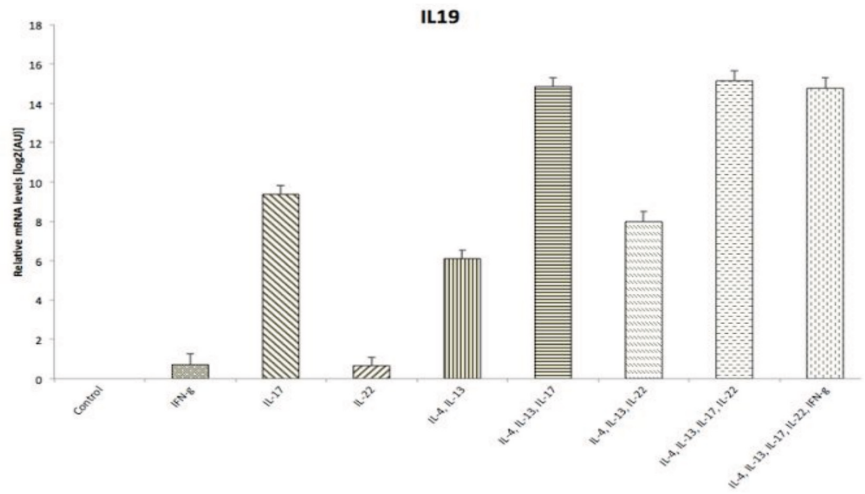


Figure 4: IL19 Expression Graph
The figure shows the relative mRNA expression levels for the gene IL19 in response to the cytokines presented. The x-axis of the graph lists the cytokines presented in the simulation. The y-axis shows the relative mRNA expression levels in Log2(AU). The graph shows that there are synergistic effects on the IL-4, IL-13 on IL-22 combinations of cytokines. There are also antagonistic effects on the IL-4, IL-13 on IL-17; IL-4, IL-13 on IL-17 on IL-22; IL-4, IL-13 on IL-17 on IL-22, IFNg combinations of cytokines.

The CXCL1 gene is a protein coding gene that codes for a chemokine in the CXC subfamily, a signaling protein secreted by cells [7]. The chemokine plays a role in inflammation and has increased chemotactic activity [7]. It is a secreted growth factor that stimulates cell growth, survival, differentiation, tissue repair, and most relevant, inflammation [7]. This protein is produced in the skin and plays an important role in inflammation as it is related to the TH17 inflammatory response [7].

The CAMP gene is a protein coding gene that codes for a member a the antimicrobial peptide family [3]. It has antibacterial, antifungal, and antiviral activities as well as a role in cell chemotaxis, immune mediator induction, and the regulation of inflammatory responses [3]. It is a protein that protects the skin from bacteria, fungus, viruses, and infection as well as controlling the inflammation in Atopic Dermatitis [3].

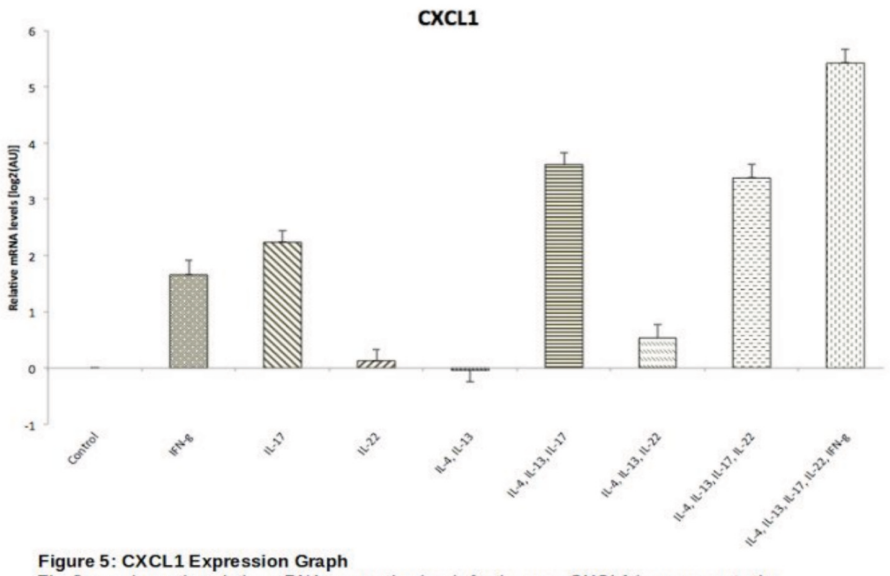


Figure 5: CXCL1 Expression Graph
The figure shows the relative mRNA expression levels for the gene CXCL1 in response to the cytokines presented. The x-axis of the graph lists the cytokines presented in the simulation. The y-axis shows the relative mRNA expression levels in Log2(AU). The graph shows that there are synergistic effects on the IL-4, IL-13 on IL-22; IL-4, IL-13 on IL-17; IL-4, IL-13 on IL-17 on IL-22; IL-4, IL-13 on IL-17 on IL-22, IFNg combinations of cytokines.

The S100A7 gene is a protein coding gene that codes for a member of the S100 protein family [26]. It is involved in the regulation of cellular processes, cell cycle progression, and cell differentiation [26]. Overexpression of this protein can cause hyperproliferative skin diseases and antibacterial activity that induces immunomodulatory activities [26]. Secreted by epithelial cells, the protein acts as a defense mechanism and is overexpressed in Atopic Dermatitis [26].

The LCN2 gene is a protein coding gene that codes for a protein in the lipocalin family [19]. It plays a role in innate immunity by preventing bacterial growth [19]. This protein is involved in cellular processes, maintain-

ing skin homeostasis, and is has decreased levels in Atopic Dermatitis [19].

The PI3 gene codes for a elastase-specific inhibitor [25]. It acts as an antimicrobial peptide against both gram positive and gram negative bacteria and fungal pathogens [25]. It is upregulated by lipopolysaccharides and cytokines. In Atopic Dermatitis, these pathways are over engaged [25].

The DEFB4B gene is a defensin coding gene that codes for members of the defensin family [9]. The defensins are small cysteine-rich cationic proteins that are microbicidal, an agent that is destructive to microorganisms, and cytotoxic [9]. This gene codes beta 4, a defensin antibiotic protein that is regulated by inflammation [9]. It comes from a family that kills microbes and is deficient in Atopic Dermatitis [9].

The CCL20 gene is an antimicrobial gene from the small cytokine CC genes subfamily [4]. They are involved in immunomodulatory and inflammatory processes [4]. Specifically, this gene works to attract inflammatory cells and chemicals to the site of inflammation [4]. In Atopic Dermatitis, the gene is overexpressed and there is too much attraction of inflammatory mediators to sites of inflammation [4].

Goals of Study/Hypothesis

In this project we tested the mRNA expression of inflammatory mediators of each immune pathway produced by keratinocytes with stimulation of different combinations of cytokines. Specifically we tested terminal differentiation genes (LOR, FLG, INV), a Th2 chemokine (CCL26), a Th1 chemokine (CXCL10), and Th17 markers (IL-19, CXCL1, CAMP, S100A7, LCN2, PI3, DEFB4B, and CXCL20). We are investigating the expression of these genes to determine how the cells are responding to various combinations of cytokines. The results will help us further understand the complex skin barrier pathways of Atopic Dermatitis.

(...)

Results

While the genes LOR, FLG, INV, CCL26, CXCL10, CAMP, S100A7, LCN2, PI3, DEFB4B, and CCL20 did not show any synergistic effects in the mRNA expression in response to the combinations of TH22 and TH17 cytokines, they still showed additive and antagonistic effects. The gene IL19 showed synergistic effects in mRNA expression in response to IL-4, IL-13 on IL-22. The gene CXCL1 showed the greatest synergistic effects with all four combinations of cytokines. These results show how certain inflammatory mediators may play a role in the skin barrier pathways of Atopic Dermatitis.

Discussion

Though these results are preliminary, they indicate promising results for the continuation of this study. The gene CXCL1 indicated all synergistic effects when the mRNA expression was tested for each cytokine as presented in figure 5. It is a generally upregulated gene with two values in which the effect of IL-4, IL-13 and IL-22 on the gene CXCL1 did not have a significant p value, making them insignificant results as presented in figure 3. The synergy may mean that the combinations of cytokines (I L-4, IL-13 on IL-17; I L-4, IL-13 on IL-22; I L-4, IL-13 on IL-17 on IL-22; I L-4, IL-13 on IL-17 on IL-22, INFg) are making the CXCL1 gene have higher mRNA expression levels in Atopic Dermatitis than in in normal skin as presented in figure 5.

The gene IL19 indicated synergistic effects when the mRNA expression was tested for oe combination of cytokines as presented in figure 4. The gene IL19 has synergistic effects with IL-4, IL-13 on IL-22 and the p value, when IL-4, IL-13 & IL-22 was tested on the gene IL19, presented a significant pvalue less than or equal to 0.005 as presented in figure 3. IL-4, IL-13 & IL-22 also showed to be upregulated by approximately .75 units as presented in figure 3. The synergy may mean that the cytokines (I L-4, IL-13 on IL-17) are making the IL19 gene have higher mRNA expression levels in Atopic Dermatitis than in in normal skin.

Mentor:
Dr. Emma Guttman , Ph.D.

PROFESSOR Dermatology; ASSOCIATE PROFESSOR Medicine, Clinical Immunology.

Mount Sinai Medical School, ICahn Medical Institute.

Synergistic, Antagonistic, Additive Results													
	LOR	FLG	INV	CCL26	CXCL10	IL19	CXCL1	CAMP	S100A7	LCN2	PI3	DEFB4B	CCL20
IL-4, IL-13 on IL-17	+	+	-	-	-	-	*	-	-	-	-	-	-
IL-4, IL-13 on IL-22	-	-	-	-	-	*	*	-	-	-	-	-	-
IL-4, IL-13 on IL-17 on IL-22	+	+	-	-	-	-	*	-	-	-	-	-	-
IL-4, IL-13 on IL-17 on IL-22, IFNg	-	-	-	-	-	-	*	-	-	-	-	-	-

Figure 6: Synergistic, Antagonistic, Additive Results
The header on the top of the table lists all of the genes tested in the study. The leftmost column lists the combinations of cytokines used in this study. IL4 and IL3 is not a combination that is tested for synergy because these two cytokines are very similar in structure and function and are considered as one cytokine. The + stands for additive mRNA expression, the - stands for antagonistic mRNA expression, and the * stands for synergistic mRNA expression. The table shows that there are synergistic effects on the IL-4, IL-13 on IL-22 combinations of cytokines on the gene IL19. There are also antagonistic effects on the IL-4, IL-13 on IL-17; IL-4, IL-13 on IL-17 on IL-22; IL-4, IL-13 on IL-17 on IL-22, IFNg combinations of cytokines on the gene IL19. The table shows that there are synergistic effects on the IL-4, IL-13 on IL-22; IL-4, IL-13 on IL-17; IL-4, IL-13 on IL-17 on IL-22; IL-4, IL-13 on IL-17 on IL-22, IFNg combinations of cytokines on the gene CXCL1.

The results for the other genes did not have any synergistic effects but had additive and antagonistic effects. Though CXCL1 and IL19 have promising preliminary results, more test must be done to further show the effect of these genes on Atopic Dermatitis. The synergistic effects tells us that the cytokines may have an affect on the genes that regulate inflammatory processes. The antagonistic effects that are shown in the mRNA expression of certain combinations of cytokines will need to be examined further to determine their roles in the skin barrier pathways. Due to the limitation of time, the microarray results have not yet been processed but once they are, we will be able to see thousands of genes' synergistic, antagonistic, and additive effects in Atopic Dermatitis to see what other inflammatory genes play a role in this disease.

Conclusion

The hypothesis was supported in this study because CXCL1 and IL19 did show synergistic effects from the mRNA expression for the different combinations of TH22 and TH17 cytokines. The synergy may mean that the combinations of cytokines (I L-4, IL-13 on IL-17;

I L-4, IL-13 on IL-22; I L-4, IL-13 on IL-17 on IL-22; I L-4, IL-13 on IL-17 on IL-22, INFg) are making the CXCL1 gene have higher mRNA expression levels in Atopic Dermatitis than in normal skin. The CXCL1 gene codes for a chemokine in the CXC subfamily, a signaling protein secreted by cells and it plays a role in inflammation and has increased chemotactic activity [7].

This protein is produced in the skin and plays an important role in inflammation as it is related to the TH17 inflammatory response [7]. This may mean that the gene CXCL1 has a direct linkage to the TH17 inflammatory pathways and possibly be a direct cause of Atopic Dermatitis.

The synergy may mean that the cytokines (I L-4, IL-13 on IL-17) are making the IL19 gene have higher mRNA expression levels in Atopic Dermatitis than in in normal skin. The IL19 gene codes for a cytokine in the IL19 subfamily, inflammatory cytokine, maintaining inflammation in Atopic Dermatitis [16]. This may mean that the gene IL19 has a direct linkage to the inflammatory cytokine pathways and possibly be a direct cause of Atopic Dermatitis. Due to limitations on time, this study's results are purely preliminary and once the microarray is completed, there will be even more genes that are tested for synergistic, additive, and antagonistic effects. This notion will help further understand the complex immune status of Atopic Dermatitis. In future studies we would like to further analyze the results of the microarray to see which other genes show synergistic effects in mRNA expression from the combinations.

Investigating the expression of these genes to determine how the cells are responding to various combinations of cytokines will help us further understand the complex skin barrier pathways of Atopic Dermatitis and may help us provide ways to treat it.

Bibliography

1. Asahina, R., & Maeda, S. (2016, May). A review of the roles of keratinocyte-derived cytokines and chemokines in the pathogenesis of atopic dermatitis in humans and dogs. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27426268

2. Belgrave, D. C.M., Simpson, A., Buchan, I. E., & Custovic, A. (2015, September). Atopic Dermatitis and Respiratory Allergy: What is the Link. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/26566461

3. CAMP Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=CAMP

4. CCL20 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=CCL20

5. CCL26 Gene (Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/

carddisp.pl?gene=CCL26

6. CXCL10 Gene (Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=CXCL10

7. CXCL1 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=CXCL1

8. Darlenski, R., Kazandjieva, J., Hristakieva, E., & Fluhr, J. W. (2014). Atopic dermatitis as a systemic disease. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24767188

9. DEFB4B Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=DEFB4B

10. Du, H.-Y., Fu, H.-Y., Li, D.-N., Qiao, Y., Wang, Q.-W., & Liu, W. (2016). The Expression and Regulation of Interleukin-33 in Human Epidermal Keratinocytes: A New Mediator of Atopic Dermatitis and Its Possible Signaling Pathway. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27348082

11. FLG Gene (Protein Coding). (n.d.). Retrieved November 6, 2016, from

http://www.genecards.org/cgi-bin/carddisp. cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. American Academy of Allergy, Asthma & Immunology. http://dx.doi.org/10.1016/j.jaci.2012.07.012

13. Hamilton, J., Suarez-Farinas, M., Dhingra, N., Cardinale, I., Li, X., Kostic, A., . . . p> Guttman-Yassky, E. (2014, December). Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. Elsevier Inc. http://dx.doi.org/10.1016/j.jaci.2014.10.013

14. Hamilton, J. D., Suarez-Farinas, M., Dhingra, N., Cardinale, I., Li, X., Kostic, A., .Guttman-Yassky, E. (2014). Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25482871

15. Ibrahim, Z. A., Ghaly, N. R., El-Tataway, R. A., Khalil, S. M., & El-Batch, M. M. (2014). A proliferation-inducing ligand in atopic dermatitis and vitiligo. Retrieved from http://onlinelibrary.wiley.com/doi/10.1111/ijd.12176/full

16. IL19 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=IL19

17. IVL Gene (Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=IVL

18. Janeway, C., Jr., Travers, P., & Walport, M. (2001). Immunobiology: The Immune System in p>Health and Disease (5th ed.). Retrieved from

http://www.ncbi.nlm.nih.gov/books/NBK27101/

19. LCN2 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=LCN2

20. Leung, D. Y.M. (2016, August). Clinical implications of new mechanistic insights into atopic dermatitis. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27191525

21. LOR Gene (Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=LOR

22. Malajian, D., & Guttman-Yassky, E. (n.d.). New pathogenic and therapeutic paradigms in atopic dermatitis. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25542094

23. Noda, S., Krueger, J. G., & Guttman-Yassky, E. (2014). The translational revolution and use of biologics in patients with inflammatory skin diseases. American Academy of Allergy, Asthma & Immunology. http://dx.doi.org/10.1016/j.jaci.2014.11.015

24. Ong, P. Y., & Leung, D. Y.M. (2016, July). Bacterial and Viral Infections in Atopic Dermatitis: a Comprehensive Review. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/27377298

25. PI3 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=PI3

26. S100A7 Gene(Protein Coding). (n.d.). Retrieved November 6, 2016, from http://www.genecards.org/cgi-bin/carddisp.pl?gene=S100A7

27. Saluja, R., Khan, M., Church, M. K., & Maurer, M. (2015). The role of IL-33 and mast cells in allergy and inflammation. Retrieved from

https://ctajournal.biomedcentral.com/articles/10.1186/s13601-015-0076-5

28. Suarez-Farinas, M., Tintle, S., Shemer, A., Chiricozzi, A., Nograles, K.E., Cardinale, I., Guttman-Yassky, E. (2011, April). Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/21388663

29. Takai, T., Chen, X., Xie, Y., Vu, A. T., Le, T. A., Kinoshita, H., . . . Okumura, K. (2014).

TSLP expression induced via Toll-like receptor pathways in human keratinocytes. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24377934

30. Tanaka, R. J., Ono, M., & Harrington, H. A. (2011, May). Skin Barrier Homeostasis in Atopic Dermatitis: Feedback Regulation of Kallikrein Activity. Retrieved from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0019895

31. Tien, K.-J., Chou, C.-W., Lee, S.-Y., Yeh, N.-C., Yang, C.-Y., Yen, F.-C., . . . Weng, S.-F. (2014, February). Obstructive Sleep Apnea and the Risk of Atopic Dermatitis: A Population-Based Case Control Study. Retrieved from http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0089656

32. Werfel, T. (2009, April). The Role of Leukocytes, Keratinocytes, and Allergen-kSpecific IgE in p> the Development of Atopic Dermatitis. Journal of InvestigativeDermatology. http://dx.doi.org/10.1038/jid.2009.71

An Epidemiological Study of Aphantasia by Melody Munitz (WESEF paper excerpt) (Junior)

Abstract

Aphantasia is a newly recognized condition characterized by the lack of visual imagery. There has been little research conducted on it. We conducted a survey over social media directed to groups of Aphantasiacs, and received 312 responses. This epidemiological study of Aphantasia is the first of its kind, providing important information about the nature of the condition and its effects on those who have it. The results from this study do not suggest a genetic cause. The results do prove that Aphantasiacs find mental workarounds to compensate for their lack of visualization skills. One of the most prominent results relates to the majority of Aphantasiacs' ability to dream visually, suggesting a different neurological mechanism of recall in Aphantasia. Another important result lies in the fact that most Aphantasiacs experience diminished imagery of their other senses, not simply visual; i.e., they have difficulty conjuring auditory or gustatory imagery. This also points to different activated brain regions in this condition, and calls for further research with an fMRI.

Introduction

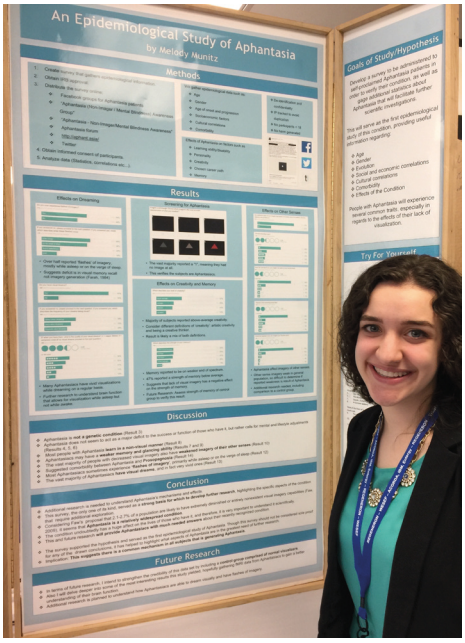
One great human ability is that of visualisation, or being able to conceive an image in one's mind's eye. Believed to be the product of fronto-parietal and posterior brain processes (Bartolomeo, 2008), this skill has numerous practical implications, such as its assistance in learning, the formation of relationships, and creativity, as well as mental state and other psychological processes. The formation of mental imagery is also linked to memory, as supported by Marks' 1973 experiment in which male and female subjects who claimed to have varying visual imagery capabilities were shown an image and then asked to recall it. The results showed that the subjects who claimed to have higher visual imagery capabilities were more accurate in their recall of the images they had seen, allowing for the conclusion that "images have an important role in memory" (Marks, 1973). In another experi-

ment relating to the correlation between eye movements and visualization, there was "no evidence that vivid visualizers showed more scanning activity than a group of Ss [patients] operationally defined to be poor at visualizing", proving that visual imagery is more than simply eye movements (Marks, D.F., 1973)

Some of the first documented research regarding visual imagery was conducted in England in 1880 by Sir Francis Galton, and was geared towards determining how different people's visual imagery skills compare. In order to assess where each patient lay on the scale of visualization, Galton developed the Vividness of Visual Imagery Questionnaire, which has since become a standard of scientifically quantifying visualization skills. This survey describes several situations and then asks the participant to rate the vividness of their mental imagery on a scale of 1-5. Through the use of the VVIQ and other similar tools and questionnaires, Galton found that not everyone is equipped with comparable visual imagery capabilities. While most people fall somewhere on the high end of the spectrum, having good or even superb visual imagery skills, there is a small percentage of people that possess very poor visualization abilities (Galton, 1880). In similar research conducted by Faw in 2009, assessing such capabilities of 2,500 participants, it was found that 2.1-2.7% of them fell in this small group, claiming extremely poor or absent visual imagination (Faw, 2009)

Until recently, aside from Faw's limited work, this phenomenon has not received much scientific exploitation. Just recently, however, Professor Adam Zeman at the University of Exeter has begun conducting research on this topic. Since named 'aphantasia' (Zeman, 2015), this condition is believed to be the result of two different types of neurogenic visual imagery impairments: i) visual memory disorders, and ii) 'imagery generation' deprivation (Farah, 1984). To explore further features of Aphantasia, Zeman and his team distributed a shortened version of the aforementioned VVIQ as well as a supplemental questionnaire addressing additional personal information to 21 participants who suspected that they had the condition, 19 of whom were male. This testing generated various statistics; 5/21 reported having relatives who experience similar symptoms, "10/21 claimed that all modalities of imagery [hearing, touch, smell, sight, taste] were affected", and that most participants claimed having realized their visualizing deficit during their teens or early twenties through conversation with 'normal' visualizers. Interestingly, despite the 21 participants' VVIQ scores being tremendously lower than those of the 121 control patients, the majority of subjects claimed involuntary imagery, 10/21 during wakefulness and 17/21 while dreaming. These statistics have helped to begin to quantify the condition of aphantasia, and are a good basis for further research, such as that I am planning to conduct (Zeman, 2015).

A questionnaire will be created and distributed via the internet to self-proclaimed aphantasia patients whose answers, upon undergoing statistical analysis, will aid in determining additional etiological statistics of the condition, including age of onset, how it is presented in both genders, and progression, as well as demographics of the condition such as any potential environmental risk factors, socioeconomic factors, or cultural correlations. Additionally, the study will consider the effects of aphantasia on learning ability/disability, personality, creativity, and chosen career path, as well as hypothesized comorbidity between aphantasia and other conditions. This data will facilitate an improved understanding of Aphantasia within the scientific community, and depending on the results of this study, further studies can be carried out in the hopes of understanding the cause of Aphantasia and possible preventative and therapeutic measures.



Goal Of Study/Hypothesis

Develop a survey to be administered to self-proclaimed Aphantasia patients in order to verify their condition, as well as gage additional statistics about Aphantasia that will facilitate further scientific investigations.

This will serve as the first epidemiological study of this condition, providing useful information regarding:

Age
Gender
Evolution
Social and economic correlations
Cultural correlations
Comorbidity
Effects of the Condition

People with Aphantasia will experience several common traits, especially in regards to the effects of their lack of visualization. (...)

Results

Result 1: Screening for Aphantasia

For each item on this questionnaire, try to form a visual image, and consider your experience carefully. For any image that you do experience, rate how vivid it is using the five-point scale described below. If you do not have a visual image, rate vividness as '1'. Only use '5' for images that are truly as lively and vivid as real seeing. For both Result 1a and Result 1b, the vast majority of participants scored their image as a "1", meaning they had no image at all. These results verify that this questionnaire was taken by people with Aphanta-

Result 1a

	1	2	3	4	5
Part a	248 / 81%	36 / 12%	9 / 3%	9 / 3%	6 / 2%
Part b	264 / 86%	26 / 8%	10 / 3%	5 / 2%	3 / 1%
Part c	248 / 86%	35 / 11%	11 / 4%	10 / 3%	4 / 1%
Part d	250 / 81%	34 / 11%	10 / 3%	8 / 3%	6 / 2%

Note: values entered as "(number of people) / (percent of people)"

Legend:

Question: "Think of the front of a shop which you often go to. Consider the picture that comes before your mind's eye."

a: "The overall appearance of the shop from the opposite side of the road."

b: "A window display including colors, shapes and details of individual items for sale."

c: "You are near the entrance. The color, shape and details of the door."

d: "You enter the shop and go to the counter. You hand the clerk your money, and they give you back change."

5 - Perfectly clear and vivid as real seeing

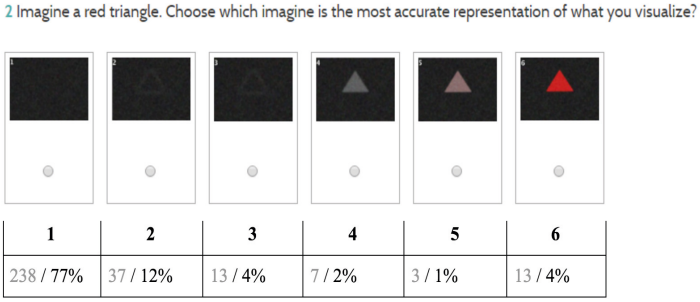
4 - Clear and reasonably vivid

3 - Moderately clear and lively

2 - Vague and dim

1 - No image at all, you only "know" that you are thinking of the object

Result 1b:



1	2	3	4	5	6
238 / 77%	37 / 12%	13 / 4%	7 / 2%	3 / 1%	13 / 4%

sia, as they reported to very low visual imagery capabilities. This is a subjective question, and especially people with a limited understanding of or limited experience with visual imagery may have a difficult time assessing their visual imagery skills.

Result 2: Age, Gender and Location of Subjects

Result 2a: There was a fair representation of age amongst study participants, with most of the participants being in the range of 25-44 years. This study was conducted with participants between a vast age range, and most ages were fairly represented.

Result 2b: There was a fairly even 55% to 45% ratio of female to male. This study did not have a bias toward one gender.

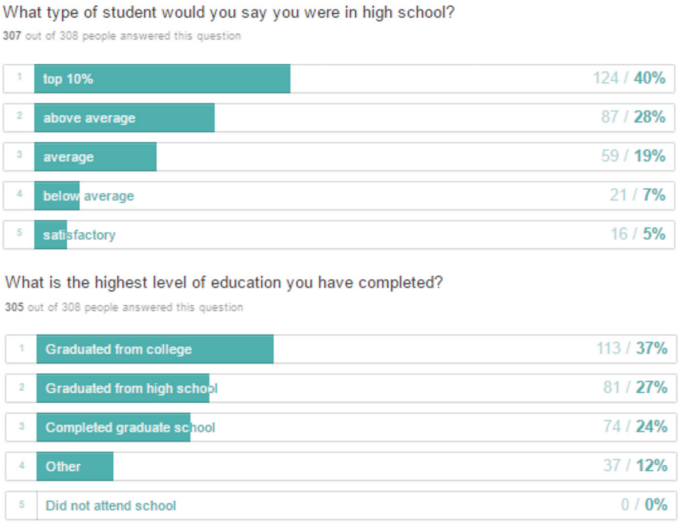
Result 2c: Question: In what country do you currently reside?

There was 40% to 60% distribution of participants residing in the United States versus other countries. It can be assumed that a large majority of the 60% in other countries are from the United Kingdom, as the sole Aphantasia researcher, Professor Adam Zeman, is in the UK, and his research has resounded especially in his region.

Result 3: Aphantasia in Blood Line

These results do not seem to indicate any any familial trait of Aphantasia. This may mean that Aphantasia is not hereditary or based on a genetic trait. Since relatives are not available for testing, this result has to be taken with caution.

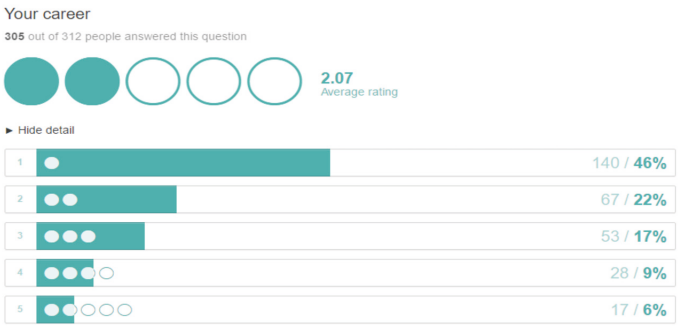
Result 4: Academic Achievement among Aphantasiacs



Most subjects were in the top 10% of their class in high school. Most subjects graduated from college. These subjects with Aphantasia do not seem to be low performing academically, in fact, they seem to be very intelligent and academically successful. Some, if not many, people would day they were better students than they may have actually been. Therefore, the bias associated with this question must be taken into consideration. Additionally, this study is a bit biased toward a group of Aphantasiacs that is inherently more educated and aware, as they recognize their condition, and have found and joined a social media group for Aphantasia.

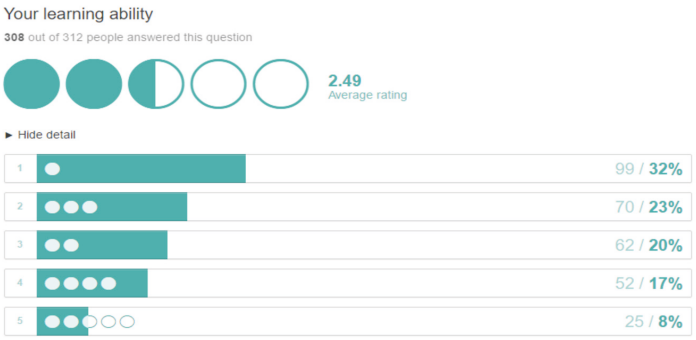
Result 5: Impact of Aphantasia

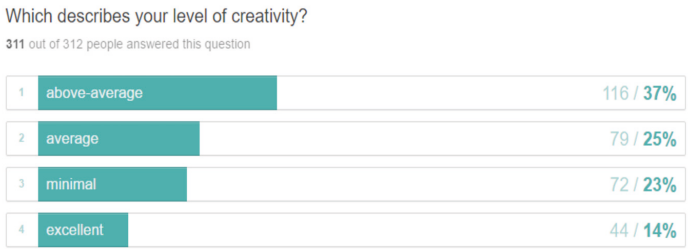
Question: "Please rate the effects of your diminished mind's eye on these various elements.



Result 6: Creativity Among Aphantasiacs

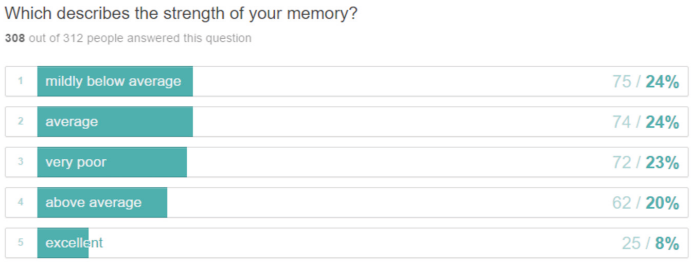
The subjects reported Aphantasia having a moderate effect on their creativity and learning ability, and a stronger effect on their memory, glancing ability, and ability to recall faces. Aphantasiacs seem to compensate for their lack of visual





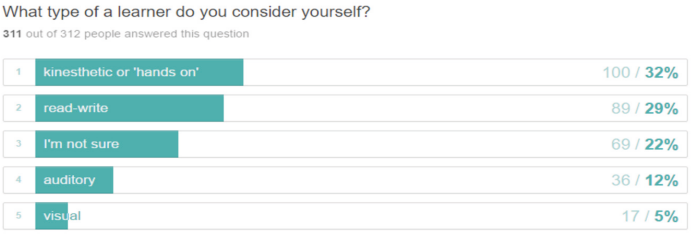
imagery and most don't see much of a deficit on their creativity or learning ability. They do however, see Aphantasia affecting their memory, glancing ability, and ability to recall faces. It is more difficult to see a means of compensation at work here among Aphantasiacs considering these are visual-dependent tasks. When assessing the inconclusive results for creativity, in which 25% of the subjects reported a "1", meaning Aphantasia had a very low effect on their creativity, and 23% reported a "5", meaning a very high effect on their creativity, the varying definition of 'creativity' must be taken into consideration; these results may either speak to artistic creativity, or to being a creative thinker (i.e. thinking in a creative manner). The latter may be more prominent among Aphantasiacs, as their possible compensation for their lack of visual imagery and ability to think or learn in that manner may mean they rely on other, 'creative', methods of thinking. Therefore, we deemed it important to have Aphantasiacs describe their level of creativity. The varying definition of 'creativity' must again be taken into consideration; these results may either speak to artistic creativity, or to being a creative thinker (i.e. thinking in a creative manner). This result of majority above-average creativity may speak to either interpretation of creativity, or more likely, a mix of both.

Result 7: Strength of Memory in Aphantasiacs



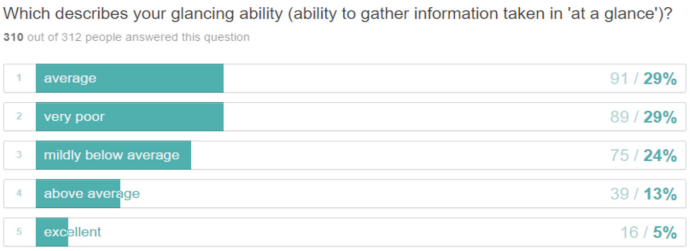
Most subjects reported that their memory is on the weaker end of the spectrum, most reporting either average, mildly below average, or very poor memory. 47% reported a strength of memory below average. This result suggests that a lack of visual imagery has a negative effect on the strength of memory. We are planning on assessing the strength of memory of non-Aphantasiacs to see if this percentage constitutes a deficit of memory in relation to Aphantasia.

Result 8: Learning Style of Aphantasiacs



Most subjects reported being either "kinesthetic" or "read-write" learners, with very few reporting to being visual learners. Considering that the deficit of Aphantasia is centered around visual imagery, it makes sense that Aphantasiacs would not likely learn visually. If Aphantasia is as common as Faw proposed (2.1-2.7%) (Faw, 2009), schools should be aware of it, and implement various learning techniques to benefit students of all types.

Result 9: Glancing Ability in Aphantasiacs



These results show that approximately the same number of subjects reported average glancing ability as reported very poor glancing ability. Very few reported having excellent glancing ability. These results allow for the conclusion to be drawn that Aphantasia typically has a negative effect on glancing ability.

Result 10: Effect of Aphantasia on All Sense Imagery

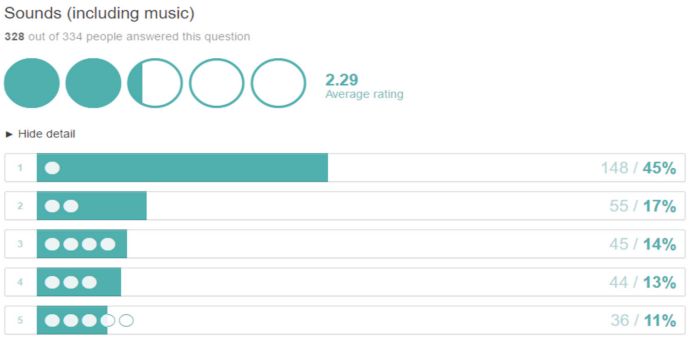
Result 10a:

The vast majority of subjects reported that they indeed have other types of decreased sense imagery. This question is aimed at determining if Aphantasia affects other types of sense imagery. These results seem to support this concept, hinting that Aphantasia may have effects beyond visual imagery.

Result 10b: Strength of Each Sense Imagery among Aphantasiacs

Olfactory: Average score, 1.54, Score of 1: 71%

Gustatory: Average score, 1.66, Score of 1: 66%



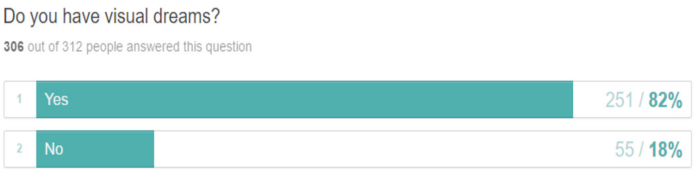
Touch: Average score, 1.87%, Score of 1: 59%

Though this study asked about the strength of all types of imagery in Aphantasiacs, not all of the results were especially telling. Because other sense imagery (auditory, gustatory, olfactory) tends to be relatively weak in the general population, it is difficult to discern if the weakness identified by the studied Aphantasiacs is the result of their condition, or is based on the fact that most people are not very strong in this regard to begin with. However, since most people have relatively strong auditory imagery, and Aphantasiacs reported relatively weak auditory imagery, this is the most telling result. This result for auditory imagery suggests that Aphantasia might affect imagery of other senses beyond visual. This is a very interesting result that calls for additional research to be able to compare it to a normal population of visualizers.

Result 12: Flashes of Imagery

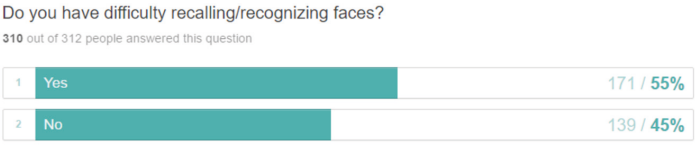
Results: The majority of the subjects, 56%, report to having flashes of imagery. 60% percent of these 56% reported visual imagery flashes while asleep, 56% reported this occurrence on the verge of sleep, 22% experience this during wakefulness, and 18% say they experience these visual imagery flashes both while awake and while asleep. A bit over half of the subjects report to having 'flashes' of imagery, mostly while asleep or on the verge of sleep. This is a very interesting set of data that requires further research to understand the brain function that allows these flashes of imagery to occur, and why specifically they are more commonly while the person is asleep. For people with a limited understanding of, or limited experience with, visual imagery, it may be difficult to recognize and categorize these 'flashes'. Therefore, these results must be considered with an understanding of the subjects whom they are gathered from.

Result 13: Dreaming in Aphantasiacs



The vast majority of subjects, 82%, reported experiencing visual dreams. Of these 82%, 39% always have visual dreams, 33% rarely have visual dreams and 28% frequently have visual dreams. When the subjects have visual dreams, 30% report them being extremely vivid, while only 12% report them being vague or lifeless. This is a very fascinating result because it shows that though Aphantasia affects visualization while awake, many Aphantasiacs seem to be having very vivid visualizations while dreaming on a regular basis. This result also calls for further research that could explain what brain function allows for visualization while asleep, but prohibits it during wakefulness. It must be recognized that for people without any visual imagery on a regular basis, even dim visual dreams may register as extremely vivid. Therefore, the results about the strength of such visual dreams must be considered in the context of the study.

Result 14: Face Recognition in Aphantasiacs



This result supports a possible link between Aphantasia and Prosopagnosia (the inability to recall or recognize faces), hinting that Aphantasia could be the result of a malfunction in the fusiform area, the region responsible for Prosopagnosia.

Discussion

This questionnaire yielded many fascinating results. Result 3 seems to weigh out the possibility of Aphantasia being a genetic condition, meaning further research should be conducted to understand its cause. Results 4, 5 and 6 show that Aphantasia does not seem to act as a major deficit to the success or function of those who have it, but rather just calls for mental and lifestyle adjustments to compensate for its effects. This claim is further supported by Result 8 that shows that most people with Aphantasia learn in a non-visual manner. Results 7 and 9 show that Aphantasia does have some negative effects, namely that Aphantasiacs may have a weaker memory and glancing ability. Result 10 provides especially fascinating results: that the vast majority of people with decreased visual imagery also have weakened imagery of their other senses. This is also connected to Result 14 that suggests comorbidity between Aphantasia and Prosopagnosia. Result 12 yielded the intriguing result that most Aphantasiacs sometimes experience 'flashes of imagery', primarily while asleep or on the verge of sleep. Result 13 adds to this concept, showing that the vast majority of Aphantasiacs have visual dreams, and in fact very vivid ones. This result definitely calls for further research to explain what brain mechanism allows for involuntary visualization while asleep, but not voluntary imagery while awake. In providing lots of unique and interesting results, this survey supported the hypothesis and served as the first epidemiological study of Aphantasia. Though this survey should not be considered sole proof for any of the drawn conclusions, it has helped to highlight what aspects of Aphantasia are in the greatest need

for further research.

Conclusion

This study was very successful, in that it received many responses and yielded many unprecedented results. Since Aphantasia is such a newly recognized condition, lots of research is needed to understand its mechanisms and effects. This survey, the only one of its kind, served as a strong basis for which to develop further research, highlighting the specific aspects of the condition that require additional exploration. In terms of future research, I intend to strengthen the credibility of this data set by including a control group comprised of normal visualizers, as well as delving deeper into some of the most interesting results this study yielded, hopefully gathering fMRI data from Aphantasiacs to gain a better understanding of their brain function. Considering Faw's proposal that 2.1-2.7% of a population are likely to have extremely diminished or entirely nonexistent visual imagery capabilities (Faw, 2009), and recognizing the high number of responses this study received in a very short period, it seems that Aphantasia is a relatively widespread and condition. Due to its nature, the condition undoubtedly has a huge affect on the lives of those who have it, and therefore, it is very important to understand it scientifically. By exploring Aphantasia, and learning more about it, Aphantasiacs will receive more answers about the bewildering and thus far unexplained condition that has such a vast impact on their lives.

References:

Bartolomeo, P. (2008). The neural correlates of visual mental imagery: an ongoing debate. Cortex, 44, 107-108.

Farah, M.J. (1984). The neurological basis of mental imagery: a componential analysis. Cognition, 18, 245-272.

Faw, B. (2009). Conflicting intuitions may be based on differing abilities - evidence from mental imaging research. Journal of Consciousness Studies, 16, 45-68.

Galton, Francis (1880). Statistics of mental imagery. Mind, 5, 301-318.

Marks, David F. (1973) "Visual imagery differences and eye movements in the recall of pictures." Perception & Psychophysics 14.3

Marks, D.F. (1973). Visual imagery differences in the recall of pictures. British Journal of Psychology, 64: 17–24.

Zeman, A., Dewar, M., & Della Sala, S. (2015). Lives without imagery: Congenital aphantasia. Cortex, 73, 378-380.

Dr. Adam Zeman, MD

PROFESSOR of Cognitive and Behavioral Neurology

University of Exeter, Medical School, Exeter, UK.

We are very grateful to Dr. Zeman for his dedication and help

The Neuroprotective Effects of Ischemic Preconditioning in an In Vitro Stroke Model, by Quinn Gruber (Junior)

Ischemic strokes, which make up the majority of strokes that occur, happen when blood flow to the brain is blocked (American Stroke Association, 2016). Strokes can cause cognitive and physical disabilities, and are the most prevalent cause of disability in the United States (Mozaffarian, 2016). Although the effects of a stroke vary depending on which region of the brain experiences the stroke, impacts may include paralysis, declined memory, speech, and cognition, and behavioral changes (Johns Hopkins University, 2017). Strokes cause many instances of death each year and cost the United States 33 billion dollars in 2011; thus, the search for a way to limit stroke damage is essential (American Heart and Stroke Associations, 2016).

Ischemic strokes cause damage in two stages: the stroke, which prevents blood reaching the brain, and during the reperfusion period, which is when blood flow is restored. Causes of ischemia reperfusion (I/R) damage are not entirely known. I/R damage has been linked to oxidative stress and accumulation of free radicals, ions which have a single unpaired electron, in turn causing inflammation and cell apoptosis. After an ischemic insult, the brain lacks necessary antioxidant defenses to protect neurons against free radical excitotoxicity, the overexcitation of glutamate amino acid receptors such as the N-methyl-D-aspartate (NMDA) receptor. Due to this antioxidant deficiency, free radicals activate apoptosis and subsequently cause neuronal death (Dugan, 1999).

One method of addressing the lack of neuronal defenses against stroke is the implementation of ischemic preconditioning (IPC), which exposes neurons to a shorter and sublethal ischemia insult before a more severe ischemia. Lee et al. found that IPC increased levels of heme-oxygenase 1, an antioxidant and antiapoptotic protein, and decreased levels of neuronal death (Lee, 2015). IPC has also been found to both increase antioxidant enzyme activity and decrease the size of cerebral infarcts from I/R (Saad, 2015).

Purpose of study: We will determine the effectiveness of IPre and IPost as treatments for ischemic neuronal damage, and underlying receptor mechanisms

Hypothesis: IPre and IPost treatments on hippocampal slice cultures protect neurons from ischemia-induced death

and reduce brain damage from oxygen-glucose deprivation.

Materials and Methods:

Researcher-performed procedures: Mice were anesthetized and sacrificed by decapitation. Hippocampi were retrieved from mice and sliced to a thickness of 400 µm using a vibratome before distribution in plates. Slices were submerged in 1 mL culture media for one week and placed in an incubator at 5.0 % CO2 and 35 °C before experiments. Plates were distributed between multiple members of the lab for use in separate experiments.

Student-performed procedures: Ischemic preconditioning and ischemic insults were simulated via oxygen-glucose deprivation (OGD). Ischemic preconditioning (IPC) was performed by placing slices in oxygen and glucose deficient media and a hypoxygenic incubator. Control slices remained in culture media and normal incubator. After OGD, slices were returned to culture media and incubator and given a 24 hour reperfusion period. After this reperfusion period, slices were dyed with 10 mL propidium iodide (PI), a fluorescent stain which permeates damaged cell membranes and binds itself with DNA, indicating cell death. The CA1, CA2, and dentate gyrus (DG) were photographed under fluorescence and under normal light. Fluorescence was measured in arbitrary units, with the average values of experimental group fluorescence levels normalized [unsure of how exactly the math works] with the average fluorescence of control slices.

Results:

There was no marked difference between the control, OGD, or IPC groups in fluorescence photos. Slices showed little levels of fluorescence across all groups, indicating an error in experimental procedure occurred, resulting in flawed results. Due to the lack of distinguishable trends in neuron death, the experiments are inconclusive.

Discussion:

Many past studies use an in vivo model to simulate ischemia, inducing ischemic insult by bilateral carotid occlusion, clamping the arteries shut to induce ischemia. The in vitro model used may not reflect an accurate model of I/R, as the culture media does not necessarily model blood flow in vivo. The experiments do not provide evidence to support the effectiveness of ischemic preconditioning; however, as the results are unreliable, ischemic preconditioning cannot be ruled out as a possible treatment of ischemia-induced injury. Since multiple studies have found positive neuroprotective effects stemming from ischemic preconditioning, more experiments are required to determine the effectiveness of IPC in an in vitro stroke model. Furthermore, one must acknowledge the unwieldiness of IPC regarding its use as a stroke treatment. Since one cannot predict exactly when a stroke will occur, IPC is not necessarily a convenient method for treating stroke damage. [add information about CAGB and how IPC can help with any possible damage from restriction of blood flow during surgery]

Conclusion:

Multiple studies have found ischemic preconditioning as an effective method of reducing damage from ischemic insult. The mechanisms of IPC are still not clear but are theorized to involve anti-inflammatory and antioxidant mechanisms which allow the brain to prepare for a more severe ischemic insult. Our experiments did not reflect the findings of other scientists which support IPC as a method of reducing damage from ischemic insult, but, due to the inconclusive nature of the results, further experimentation is required to determine the impact of IPC on ischemic injury. Future experiments will focus upon isolating specific neural mechanisms which may protect the brain from ischemia-induced damage, and improving the slice culture model so that ischemic injury is more accurately represented.

References

American Heart Association, & American Stroke Association. (2016). Ischemic Strokes (Clots). Retrieved January 3, 2017, from http://www.strokeassociation.org/STROKEORG/AboutStroke/TypesofStroke/IschemicClots/Ischemic-Strokes-Clots_UCM_310939_Article.jsp#.WGv0HFMrKYk

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M.,Stroke Statistics Subcommittee. (2016). Heart Disease and Stroke Statistics—2016 Update. Circulation, 133(4), e38–e360. http://doi.org/10.1161/CIR.0000000000000350

Johns Hopkins University. (2016). Effects of Stroke | Johns Hopkins Medicine Health Library. Retrieved January 9, 2017, from http://www.hopkinsmedicine.org/healthlibrary/conditions/nervous_system_disorders/effects_of_stroke_brain_attack_85,P00777/

American Heart and Stroke Associations. (2016). Heart Disease and Stroke Statistics--2016 Update. Retrieved January 4, 2017, from http://www.heart.org/HEARTORG/General/Heart-and-Stroke-Association-Statistics_UCM_319064_SubHomePage.jsp

Dugan LL, Choi DW. Free Radicals in Hypoxia-Ischemia. In: Siegel GJ, Agranoff BW, Albers RW, et al., editors. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Philadelphia: Lippincott-Raven; 1999. Available from: https://www.ncbi.nlm.nih.gov/books/NBK28241/

Mentor:
Dr. Patric K. Stanton, Ph.D.

PROFESSOR of Cell Biology and Anatomy
New York Medical College, Valhalla, NY

We are grateful to Dr. Stanton for his help.

Using Bioinformatics to Interpret Epigenetic Research Data, by Peter Manthey (Junior)

Bioinformatics is an emerging discipline that uses computer technology, statistics, mathematics, and engineering to analyze and translate biological and genetic data, as well as healthcare information. Although bioinformatics has been around since the 1960s, it is now being widely used. In the simplest form, bioinformatics is data that is collected from specific sources, run through unique code and organized by the associated biological macromolecules.

The amount of data being collected today throughout the world is enormous. One of the largest databases being used for bioinformatics is the GenBank, which is part of the International Nucleotide Sequence Database Collaboration. As of December 2015 this database contained over 203 billion nucleotide bases in more than 189 million sequences, collected from daily exchanges of data with the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at NCBI.

"This database is produced and maintained by the National Center for Biotechnology Information (NCBI) as part of the International Nucleotide Sequence Database Collaboration (INSDC). The National Center for Biotechnology Information is a part of the National Institutes of Health in the United States. GenBank and its collaborators receive sequences produced in laboratories throughout the world from more than 100,000 distinct organisms." [1] As a result, there has been an increase in the application of bioinformatics tools to help medical researchers not only analyze larger quantities of data in shorter period of time, but to also become more precise in diagnosis and determining treatments. One breakthrough is using DNA sequencing to break apart a DNA strand which is made up of four nucleotide bases. By doing this scientists can determine variations for genetic diseases. Collecting all of this data and using bioinformatics will now allow doctors to break down a person's entire human genome in one day and provide personalized treatment and medication. One bioinformatics software tool currently being tested, will automate the interpretation of the genome data by accessing any medical journal, research and articles applicable to the data and allow the treating doctor to reference those sources for potential treatment.

In 2003, researchers from several esteemed universities in the United States leveraged CompuCell, a multi-model framework for the simulation of morphogenesis, to simulate the formation of limbs in multicellular organisms during their embryonic stages of development. The program was designed to help researchers better understand the processes of morphogenesis. The program simulation models the interactions between the gene regulatory network and genetic cellular mechanisms. This new technology for modeling morphogenesis could help us to better understand defects that occur during limb development.[2] In 2005 additional research was performed presenting CompuCell3D, a three-dimensional, cell-centered, multiscale framework.[3]

Today CompuCell3D is being used to in an open source environment that allows for "virtual tissue simulations of development, homeostasis, toxicity and disease in tissues, organs and organisms, covering subcellular, multi-cell and continuum tissue scales."[4]

In October of 2015, a group of scientists in Italy developed a bioinformatics program that is capable of identifying potential biomarkers for diseases and disorders know was SANIST. In this study, researchers used SANIST to identify a biomarker for prostate cancer known as carnitine, an ammonium compound involved in fatty acid metabolism. The research found that canitine was expressed at lower levels in the plasma of prostate cancer patients and concluded that SANIST was able to accurately identify and separate individuals with prostate cancer from those with benign conditions at a rapid rate.[7]

In late October of 2015 several databases have were created to provide doctors with access to allergen lists, protein classifications and sequence information to be used to determine potential risk of allergenic cross-reactivity. Over 55% of the US population has tested positive for some type of allergy. 8 Allergies are caused by a person's immune system which mistakes an allergen, a foreign body, as a threat and launches and attacks to neutralize it. The researchers used bioinformatics to predict which proteins in parasitic worms would cause a reaction similar to an allergic reaction in humans. They were able to isolate in a parasitic worm one of the most common proteins in pollen. This protein was similar to a protein only known previously in the genomes of plants. Pollen is one of the most prevalent allergens. This bioinformatics tool will allow scientists to predict proteins that cause allergies, and to design protein molecules for treating them. [9]

Right now research is occurring to find a more accurate detection for ovarian cancer in women. Currently fifty

percent of women who have been diagnosed with ovarian cancer do not survive more than 5 years. The reason this cancer is so deadly is because ovarian cancer is very hard to detect, with signs only presenting themselves normally during later stages of development. In 2012, there were over 200,000 documented cases and 125,000 deaths worldwide. Researchers have found that NSC 319726, a small-molecule anticancer, could be used to effectively treat ovarian cancer. This study used bioinformatics to analyze and map the interactions between differentially expressed genes across a network. This allows researchers to find potential targets for NSC 319726. The result suggested that these genes and pathways may be candidate agents for NSC 319726. This is because NSC 319726 has been found to reduce levels of RPS6KA6, a chemical that is found to be overexpressed in patients with cancer due to it influencing the growth of cancer cells.[10]

One of the most important tools in the CompuCell3D program is the chemical field tool. This tools allows researchers to simulate the morphogenesis of multicellular organisms. The "French flag" model displayed how cells placed into varying positions on the chemical field changed their parameters including target volume, shape, orientation and diffusion due to the surrounding environment. One prominent theory surrounding the development of a curved bird beak is that at a certain point in the beaks development, the cartilage bar will pass through a chemical field that weakens the strength of the bar. This chemical field is stronger though at the bottom of the bird's beak than it is on the top of the beak causing it to develop a hooked shape.

Recent research on the formation of body segments (somites) in vertebrate embryos also utilizes cell growth with multiple parameters dependent of the age of the developing cells. This research used bioinformatics to prove it is possible to model cells with multiple age dependent parameters in the CompuCell3D program by using local cell interactions versus an external segmentation clock. The research concludes that somites are self-organizing structures whose size and shape is controlled by local cell-cell interactions.

Over the last 10 years there has also been significant progress in the field of using bioinformatics for epigenetic research. In 2010, Bare Bones Pattern Formation: A Core Regulatory Network in Varying Geometries Reproduces Major Features of Vertebrate Limb Development and Evolution used bioinformatics to "simulated the behavior of the core chondrogenic mechanism of the developing limb in the presence of an FGF gradient using a novel computational environment that permits simulation of LALI systems in domains of varying shape and size." This research concluded when a gradient is present the limb it affects the behavior of the mesenchyme and can form limb-like skeletal structures. Bioinformatics was applied to simulate in vitro and in vivo limb development. This will allow doctors to better understand limb development and any complications that may occur in utero.

In 2012 in the Physico-Genetic Determinants in the Evolution of Development scientists found embryos exhibit an assortment of stereotypes and patterns during development that have been present for millions of years. This has lead researchers to believe the origins of animal development lay in the effects of external forces on how these animals develop. Dr. Stuart Newman believes "that the origins of animal development lay in the mobilization of physical organizational effects that resulted when certain gene products of single-celled ancestors came to operate on the spatial scale of multicellular aggregates".

Researchers compared the embryological processes that shapes the limb bud, teeth and beaks to current theory of bone and cartilage development. Bioinformatics was used to determine that the current theory only applies to limb development. This research has been the foundation for a new therapy regarding the process of evolution in which an organism's genetic expression or phenotype, will often change before there is an actual change in the organism's genetic code of genotype. This theory has more recently been used to examine the evolutionary changes that were first observed by Darwin during his finch study in the Galapagos Islands. Researchers were able to find that by introducing different external factors into the environments of a developing bird embryo, all beak shapes described by Darwin in his studies could be achieved.

Currently, cancer is the leading cause of death worldwide, with 1.65 million cases diagnosed and 600,000 deaths caused by the disease in United States alone. By using information base off of the Glazier-Granier-Hogeweg (GGH)-based model, which is used to explain how different cells locally interact with each other in an organism, research have been able to discover a

Mentor:
Dr. Stuart A. Newman, Ph.D.

PROFESSOR of Cell Biology and Anatomy

New York Medical College, Valhalla, NY.

We are grateful to Dr. Newman for his help

method for identifying cells that are either cancerous, or are at risk of becoming cancerous based upon how they interact with other surrounding cells in the organism.

It is evident that bioinformatics is a powerful tool in the evolution of predicting and treating diseases. In this research we will focus on using bioinformatics and the CompuCell3D simulation software to more accurately model bird beak development during early stages of fetal development. We will present results from numerical biologically plausible simulations that demonstrate that the development of the upper and lower beak grow independent of each other. This will be done by demonstrating the significant shape differences between control and VPA-treated embryos at each stage. Success will be determined if the simulation produces gaps or margins of error in the output graphics that parallel those observed during original study.

Methodology

Celldraw.bat is used to create the initial cell layout.

Twedit++ is used to define the cell properties and behaviors, and edit the code.

IDLE (Python GUI) is used to write scripts for more advanced cellular functions

CompuCell3D is used to run the simulation and produces output graphics

Windows Movie Maker is used to edit the output still graphics into an animated simulation.

The primary step is to establish a baseline by creating a simulation that modeled the development of the control specimen in the Limb Tooth Beak Research. Also collected algorithms from other papers published using the CompuCell3D program such as "Searching a multicellular model to tame tumor-induced angiogenesis" to aid in the creation of the CompuCell3D simulations.

References

1 GenBank Home. Retrieved January 10, 2016, from http://www.ncbi.nlm.nih.gov/genbank/.

2 Izaguirre, J. A., Chaturvedi, R., Huang, C., Cickovski, T., Coffland, J., Thomas, G., et al. (2004). CompuCell, a multi-model framework for simulation of morphogenesis. Bioinformatics, 20(7), 1129-1137.

3 Cickovski, T. M., Huang, C., Chaturvedi, R., Glimm, T., Hentschel, H. G. E., Alber, M. S., et al. (2005). A framework for three-dimensional simulation of morphogenesis. Computational Biology and Bioinformatics, IEEE/ACM Transactions on, 2(4), 273-288.

4 (2015). Compucell3d.org - Userexp.io. Retrieved January 24, 2016, from http://userexp.io/compucell3d.org.html.

5 Home - Autism Society. Retrieved January 10, 2016, from http://www.autism-society.org/.

6 (2015). $9 million grant to establish open-access autism database ... Retrieved January 10, 2016, from https://med.stanford.edu/news/all-news/2015/06/9-million-grant-to-establish-open-access-autism-database.html.

7 Albini, A., Briga, D., Conti, M., Bruno, A., Farioli, D., Canali, S., et al. (2015). SANIST: a rapid mass spectrometric SACI/ESI data acquisition and elaboration platform for verifying potential candidate biomarkers. Rapid Communications in Mass Spectrometry, 29(19), 1703-1710.

8 (2010). Allergy Statistics and Allergy Facts - WebMD. Retrieved January 10, 2016, from http://www.webmd.com/allergies/allergy-statistics.

9 Tyagi, N., Farnell, E. J., Fitzsimmons, C. M., Ryan, S., Tukahebwa, E., Maizels, R. M., et al. (2015). Comparisons of Allergenic and Metazoan Parasite Proteins: Allergy the Price of Immunity. PLoS Comput Biol, 11(10), e1004546.

10 Xue, J., Yang, G., Ding, H., Wang, P., & Wang, C. (2015). Role of NSC319726 in ovarian cancer based on the bioinformatics analyses. OncoTargets and therapy, 8, 3757.

Zhu, J., Zhang, Y., Alber, M. S., & Newman, S. A. (2010). Bare bones pattern formation: a core regulatory network in varying geometries reproduces major features of vertebrate limb development and evolution. PLoS One, 5(5), e10892.

Newman, S. A. (2012). Physico-genetic determinants in the evolution of development. Science, 338(6104), 217-219.

*Linde-Medina, M., & Newman, S. A. 2014). Limb, tooth, beak: Three modes of development and evolutionary innovation of form. Journal of biosciences, 39(2), 211-223

Epigenetics predicting our Future, by Kimberly McKoy (Sophomore)

Epigenetics are heritable changes in a gene sequence (Simmons, 2008). Epigenetics withholds a person's future as it has the power to switch genes on or off (Simmons, 2008). Methylation is the function of methyl groups being added to DNA at the CpG sites. Methylation can turn off the protective suppressor genes (Simmons, 2008). These genes are extremely important as they protect the cell from developing tumors. The tumor suppressor genes can be silenced when the DNA is methylated. In a patient, this can cause cancer (Simmons, 2008). Epigenetics is a process, which is triggered by certain environmental conditions and lies between the genotype and the phenotype (Deichmann, 2016). The genotype are all the genes in a patient. The phenotype is the expression of genes that expresses a person's height, weight and skin color, this is important as it develops who we are as people. In addition, biochemical pathways of the cell are determined by the presence of activated genes (Deichmann, 2016). The methylation patterns, which may silence certain genes, are passed from the parent to the offspring. (Deichmann, 2016). This shows us the importance of methylation of how expressed genes in the generation of grandparents can be silenced in the next generation.

The mother's environment can cause trauma to her offspring, this development is based on the nurturing of the parents (Turcot, 2017) . With the lack of nurturing, the offsprings genes can be altered by epigenetics and can change many of the brain's and body's function, causing a mood disorder in the offspring (Turcot, 2017). The depression in the mother can transform into adult depression and stress management for her offspring (Turcot,

2017). Stress can create future diseases such as neurological diseases, by modifying gene expression of the serotonin

transporter (5-HTT) influencing depressive responses in patients (Szyf, 2015 A). The genetic variation, polymorphism, is associated with the deficits in neurobehavioral functioning during infancy (Szyf, 2015 A). There are many epigenetic signatures of child abuse that could be easily identified by looking at gene expression. Genes that are silenced by methylation caused by physical abuse can affect bodily functions (Cecil, 2016). This includes the important functions that our body brings such as cardiovascular function, fear response, and wound healing (Cecil, 2016). As a result, there is a risk of heart disease among individuals who were physically abused as children (Cecil, 2016). Maltreatment has been consistently associated with these diseases and there is a reduced volume of the corpus callosum in the prefrontal cortex and hippocampus (Cecil, 2016). This corpus callosum is responsible for joining two parts of the brain together with fibers. If methylation can silence some genes preventing functions, What else can it change?

Modifications of the serotonin transporter (5-HTT) can change the behavioral responses in children. Such modifications can happen in the womb of their mothers and are influenced by environmental circumstances. Early trauma can cause changes in the precursor of thyroid-hormone T4 and T3 (Machado, 2015) . Thyroid glands take the iodine that is in food and convert it into thyroid hormones. Changes in thyroid hormones can cause changes in metabolism . As early trauma impacts the response to stress, it affects the digestive systems and other systems in the body, causing an abnormal metabolism in a patient (Machado, 2015). This is even more pronounced in victims that have already been exposed to child abuse.

The hypothalamic-pituitary adrenal axis (HPAA) has a function to send messages from the adrenals, the pituitary and other organs. Depression is linked to the dysregulation of the HPAA and this is because too much cortisol can create an imbalance in the axis causing the dysregulation (Farrell, 2016). Early exposure to stress links to the dysregulation in the altered levels of the glucocorticoid receptor and can cause depression (Farrell, 2016). Cortisol and the glucocorticoids are attached onto the glucocorticoid receptor. Glucocorticoids are hormones that are involved in the metabolism of carbohydrates, proteins and fats. Depressive disorder and the hyperactivity of the HPAA are linked to the modification of (NGFI-A), proteins that are in the nucleus (Farrell, 2016). This protein activates the EGR (early growth response) in adolescents.

Child abuse is not just about the physical damage that is affected. Genes are affected as well, just like NR3C1 gene, which codes for the stress receptor called cortisol (Eleanor, 2014). Cortisol is a stress hormone that helps you respond to threat and danger (Eleanor, 2014). Children that deal with abuse develop high levels of cortisol and develop low threshold stress levels (Eleanor, 2014). Abuse affects the organs of the body such as the hypothalamus, pituitary glands and adrenal glands (Yong, 2009). All three of these organs create hormones that control reactions to stressful situations and trigger psychological changes (Yong, 2009). The NR3C1 produces a protein called the glucocorticoid receptor, which causes a reaction that deactivates the HPAA (Yong, 2009). A deactivation of the HPAA causes a continuation of stress response which can cause someone to have high blood pressure and other nutrition problems.

There is a decreased hippocampal hGR1f (Hippocampal Glucocorticoid Receptor) expression, and the human homologue in abused suicide victims compared to nonabused suicide victims (Labonte, 2012) . The decrease in the hGR1 expression associates with the reduced HGR messenger RNA with a promoter hypermethylation (Labonte, 2012). Hypermethylation is the increasing in methylation of DNA which promotes excessive stress levels. The HGR and its noncoding exon 1B, 1C and 1H is decreased in the hippocampus of abused suicide victims (Labonte, 2012).

Childhood trauma affects many bodily functions and as an adolescent trauma can turn into something more like PTSD. Childhood trauma is determinant of psychiatric disorders and in PTSD patients, which makes the brain more vulnerable to trauma (Kim, 2016). Understanding how Epigenetic influences psychiatric illness can improve many therapeutic strategies (Kim, 2016). Extreme stress during childhood can lead to PTSD, this is formed by the excessive amount of cortisol, and many bodily functions not being able control it forming the symptoms of PTSD (Zhao, 2013). This leaves epigenetic marks that controls DNA methylation that can change the gene expression of the patient (Zhao, 2013). 98% of genetic changes in childhood abuse are the same changes in gene expression in PTSD patients (Zhao, 2013).

PTSD affects the gene expression and brain function during the development in the central nervous system (Zannas, 2015) . PTSD is linked with immune function, trauma exposure which may bring disturbance to the HPAA; since it sends important messages to the brain (Zannas, 2015). Immune dysregulation in the periphery can contribute to the development of PTSD in brain function (Zannas, 2015). Immune dysregulation is the weakness in a patient's immune function and immune system. PTSD causes the alteration in the nuclear factor Kappa B, and this factor regulates inflammatory gene networks (Guardado, 2016). As a result, there is a decrease in methylation of genes in immune and inflammatory function in PTSD patients (Guardado, 2016). Inflammatory function is the function of the body repairing cells and developing tissue repair. PTSD increases the nuclear factor kappa B which causes stress-induced inflammation and cognitive impairments which gives PTSD the symptoms that it has (Guardado, 2016).

Ancestral experiences are copied in the epigenome for

human behavior in health and diseases (Szyf, 2015 B) . Nongenetic transmissions could be triggered in responses to environmental exposure due to epigenetics (Szyf, 2015 B). When the phenotypic response to the ancestral parental experience creates generations of physical environments that were triggered by a multigenerational transmission (Szyf, 2015 B). Epigenetics predicts our future by our genes affecting many functions by the unexpected silencing of methylation that each of us have to deal with.

References

Cecil, Charlotte A.M. , Rebecca G. Smith, Esther Walton, Jonathan Mill, Eamon J. McCrory, Essi Viding. Epigenetic signatures of childhood abuse and neglect: Implications for Psychiatric vulnerability. Journal of Psychiatric Research 83 (2016) 184-194 http://dx.doi.org/10.1016/j.jpsychires.2016.09.010

Farrell, Chloe. Veronica O'Keane. Epigenetics and the glucocorticoid receptor: A review of the implications in depression. Psychiatry Research 242 (2016) 349-356http://dx.doi.org/10.1016/j.psychres.2016.06.022

Guardado, Pedro. Anlys Olivera, Heather L. Rusch, Michael Roy, Christina Martin. Natasha Lejman, Hwyunhwa Lee, Jessica M. Gill .Altered gene expression of the innate immune, neuroendocrine, and nuclear factor-kappa B (NF-ϰB) systems is associated with posttraumatic stress disorder in military personnel.Journal of Anxiety Disorders 38 (2016) 9-20 http://dx.doi.org/10.1016/j.janxdis.2015.12.004

Hafner, Sophia J. Lund, Anders H.Great expectations – Epigenetics and the meandering path from bench to bedside. Biomedical Journal 39 (2016) 166- 176 http://dx.doi.org/10.1016/j.bj.2016.01.008

Kim, Ji Sun. Lee, Seung-Hwan. Influence of interactions between genes and childhood trauma on refractoriness in psychiatric disorders. Progress in Neuro-Psychopharmacology & Biological Psychiatry 70 (2016) 162-169 http://dx.doi.org/10.1016/j.pnpbp.2016.01.013

Labonte, Benoit, Volodymyr Yerko,Jeffrey Gross, Naguib Mechawar, Michael J. Meaney, Moshe Szyf, and Gustavo Turecki. " Differential Glucocorticoid Receptor Econ 1B,1C and 1H
Expression and Methylation in Suicide Completers with a History of Childhood Abuse"(2012)

Machado, T.D . G.A Salem, V.L. Boss, M.Z. Gold and, M.J. Meaney, M. Arganonik, G.G Manfro, P.P. Silveira. Early Trauma is associated with decreased peripheral levels of thyroid-hormone T3 in adolescents. Int. J. Devl Neuroscience 47 (2015) 304-308 http://dx.doi.org/10.1016/j.ijdevneu.2015.10.005

Nelsen, Eleanor. 30 July 2014.Abuse Casts a Long Shadow by Changing Children's Genes

Simmons, D. (2008) Epigenetic influence and disease. Nature Education 1(1):6

(A)Szyf, Moshe. Epigenetics, a key for unlocking complex CNS disorders? Therapeutic Implications. European Neuropsychopharmacology (2015) 25, 682-702 http://dx.doi.org/10.1016/j.euroneuro.2014.01.00

(B) Szyf, Moshe. "Nongenetic inheritance and transgenerational epigenetics." (2015)

Turcot ,Andree-Anne Bouvette-. Unternaehrer, Eva. Gaudreau, Helene. Lydon, John E. Steiner, Meir. Meaney,Michael. The Joint contribution of maternal history of early adversity and adulthood depression to socioeconomic status and potential relevance for offspring development. Journal of Affective Disorders 207 (2017) 26-31

Ute Deichmann. Epigenetics: The origins and evolution of a fashionable topic. Developmental Biology 416 (2016) 249-254. http://dx.doi.org/10.1016/j.ydbio.2016.06.005

Zannas S., Anthony, Nadine Provencal, and Elisabeth B. Binder. " Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges,and Future Directions". (2015)

Zhao,Roseanne."Child abuse leave Epigenetic marks" (2013)

Yong, Ed. February 22 2009."Child Abuse permanently modified stress genes in brains of suicide victims"

The analysis of visual elements required for reading improvement for dyslexics by Hailey Kissner (Sophomore)

The formal definition of Dyslexia is: "a specific learning disability that is neurological in origin. It is characterized by difficulties with accurate and/or fluent word recognition and by poor spelling and decoding abilities. These difficulties typically result from a deficit in the phonological component of language that is often unexpected in relation to other cognitive abilities and the provision of effective classroom instruction. Secondary consequences may include problems in reading comprehension and reduced reading experience that can impede the growth of vocabulary and background knowledge" (The International Dyslexia Association, 2013).

The article by Lapkin, et al. describes the symptoms of dyslexia including issues with math, reading and writing and its psychological impact on an individual. For example, dyslexics struggle with issues of self-esteem, depression, and social insecurities. "A good way to understand dyslexia is to establish what it is not. It's not a sign of low intelligence or laziness. It's also not due to poor vision. It's a common condition that affects the way the brain processes written and spoken language" (Lapkin, n.d.). Dyslexia is a learning disability characterized with having issues in speech, writing, reading, and spelling. Often it takes dyslexics more time to understand complicated topics and information may need to be processed differently. This condition is claimed to be caused by hereditary traits and differences in brain anatomy. The process of early diagnosis with this disorder is key, the earlier the better because with time they can learn how to cope with dyslexia and how it impacts daily life. Although, dyslexia cannot be outgrown or cured many teaching strategies using a multisensory approach and various other tools can improve life for a dyslexic. Individuals with dyslexia experience an immense amount of hardship because their problem with reading causes low self-esteem and frustration. Therefore, dyslexics are forced to identify what different tools and techniques aid their learning process. Also, dyslexics have difficulty articulating what they want to say, challenges with memory, maintaining focus, and difficulty with organization and time management.

Despite adversity, many creative minds with dyslexia have become famous such as Tim Tebow, a football star and Dav Pilkey, the author and illustrator of the "The Adventures of Captain Underpants" series (Lapkin, n.d.).

More than 100 years ago during the November of 1896 in Sussex England, Dr.W. Prin-

gle Morgan published the first description of dyslexia a "Percy F., . . . aged 14,... has always been a bright and intelligent boy," wrote W. Pringle Morgan in the British Medical Journal, "quick at games, and in no way inferior to others of his age. His great difficulty has been--and is now--his inability to learn to read" (Shaywitz,1996). From 1896 to 1996 dyslexia was believed to be a substitute for intelligence because "only smart people could read"(Shaywitz,1996). During the 1920's scientists thought that dyslexics had a combination of issues with the body's visual system and a reading disorder, often eye training was recommended to help the alleged visual deficits. Later on, the visual issues in dyslexia were disproved and we now know that this condition is correlated to the language system. Shaywitz gives an example of the struggles of med student, Gregory, who was diagnosed with developmental dyslexia in elementary school. As a result, he received support and excelled in a program for gifted students. Later on in his life, Gregory attended an Ivy League college where he worked tirelessly in order to get into medical school. There Gregory struggled learning long words and novel terms like labels related to the structure of the human body. A new model of dyslexia was created based off phonological processing, that supports both neurological and clinical symptoms of his reading disorder. In language processing, the words are decoded in the phonological area of brain before being read. This system of spoken language is involuntary because reading can be learned at a conscious level. Gregory struggling to read long words and novel terms relates to the phonological deficit in dyslexia. A study done in the 1980's showed that a preschoolers phonological ability predicts later skills at reading. Additionally, the finding showed that remedial training intervention correlated with phonological awareness improved the children's ability to read (Shaywitz,1996).

Scientists believe children with developmental dyslexia are made up of three subtypes, rapid automatic naming (RAN), phonological and double deficit consisting of both RAN and phonological. In order to prove the double deficit hypothesis various research projects have been conducted. For instance, researchers have ested adolescents in areas relating to the specified subtypes through color naming, timed reading and math expression problems etc. During the memory tasks, there was no major difference between dyslexics and the control who did not have have a learning disability. Children presumed with the double deficit hypothesis scored significantly lower in the reading and word decoding tasks. Additionally, dyslexics completed less math problems during the math fluency and calculation tasks. Finally dyslexics had a slower performance in the color naming and digit matching tasks (Träff, 2016). The study, "Functional neuroanatomical evidence for the double-deficit hypothesis of developmental dyslexia". uses a functional MRI to learn about the double deficit hypothesis. The group of poor readers and normal readers in the study, uses tests such as phonological awareness and rapid automatized naming while a preforming a with a fMRI. Knowing that the group of individuals "showed dissociation between brain regions that were sensitive to phonological awareness (left inferior frontal and inferior parietal regions) and rapid naming (right cerebellar lobule VI)"(Norton, 2014). According to behavioral measures it is displayed that the double deficit group scored based upon their the lowest on reading ability, the system in which the tasks were scored was based on the individual's performance. There was little difference between the reading ability according to the testing of the double deficit and single phonological deficit (Norton, 2014).

Unlike developmental dyslexia which is obtained early in life, acquired dyslexia could form at any time because it is caused by damage to the brain. A study done by researchers at John Hopkins University found possible theories relating to reading in acquired dyslexics. A 69 year old patient with a ruptured cerebral aneurysm resulting in damage to the left hemisphere of her brain, has slow and inaccurate reading after her incident (Johns Hopkins University, 2010). By testing the patient, scientists found more support for allographs, two or more different forms of a letter or patterns of letters forming a sound in a word during prelexical reading which claim that allograph representations are specific, in other words,they are located at the beginning and end of the word ignoring how long it is. This provides an explanation for the patient's continuous errors because when reading, the allographs remained in their positions and mistaken for a word later on . From this study scientists also learned that the beginning and the end of the word are related to standard word orientation compared to spatial properties like up and down (Johns Hopkins University, 2010).

A study from the Massachusetts Institute of Technology uncovered that dyslexics have problems with voice recognition. In the trial, individuals with and without dyslexia identified cartoon character voices in English and Mandarin with a computer screen displaying multiple cartoon characters and had to match the voices to the avatars on the screen. The research showed that the individuals without dyslexia could correctly match the cartoons voices in English to the avatars on the computer screen 70 percent of the time and 50 percent of the time they could properly match the Mandarin speaking cartoons with their avatar. However, dyslexics were correct at matching the voices with the cartoon character in both languages only 50 percent of the time. Further proving the theory that dyslexics have difficulties with identifying voices (Belluck, 2016).

According to a study done by a professor at Harvard University, dyslexia is presumed to be correlated with the structure of white and grey matter in the brain. When an individual is reading a book the eye

takes in information which is then transported to the brain, there the regions of the brain process what is being read.. White matter, contains axons, long fibers, connecting neurons whose cell bodies are located in grey matter, allowing the essential process of connecting graphemes and phonemes. Additionally, white matter is responsible for reading development in the brain (WETA, 2015). A phoneme is a sound of a word which makes up graphemes, the written representation of a word. In language processing, the words are decoded in the phonological area of brain before being read when reading aloud (Shaywitz,1996). However, in dyslexia, parts of the brain responsible for reading are not properly connected resulting in issues with reading fluency (Nasr, 2007).

In the article "Dyslexia and the Brain: What Does Current Research Tell Us?" the authors describe dyslexics have less brain matter in their left parietotemporal area, which is responsible for verbal memory by allowing the brain to remember numbers. The authors suggest that a lack of brain matter can cause issues with phonological awareness and auditory processing. Additionally, dyslexics usually have a lack of white matter causing different regions of the brain to have difficulty communicating with each other. Also, according to brain imaging studies, right handed individuals with dyslexia have a bigger left hemisphere of their brain than right in non-dyslexics. This symmetrical difference is being studied currently; researchers suspect that it is associated with issues with reading and spelling. The article references a similar study which compared right handed dyslexics and right handed non dyslexics (control group) brain activity during reading tasks. The results demonstrated the control group had more brain stimulation while performing these tasks. This research also found that individuals who were skilled at decoding during reading had more activation in the left side of the brain and right handed dyslexics had greater activity in their frontal lobe when performing tasks (WETA, 2015).

Ihe article "Further adventures of the incredible plastic brain", FMRI scans were taken before and after remedial training in 7 to 12 year olds with and without developmental dyslexia. This training taught them to correlate consonants and vowels to sounds. As a result of the training, the children experienced more brain activation and had an easier time performing language and reading tasks. For instance, the scans of dyslexic children showed stimulation on the left side of the brain in the left parietal cortex and left inferior frontal gyrus. In comparison, non-dyslexic children had less stimulation in those specific areas in the brain. Finally, the post remedial training FMRI scans in dyslexics showed that they had activation in the right-hemisphere frontal an area of the brain responsible for nonverbal tasks, temporal regions, responsible for verbal tasks and anterior cingulate gyrus , playing a role in cognition and emotions, where they never had activity before. Meanwhile, non-dyslexics had little brain function in those areas . With vital remedial training dyslexics can further improve their reading skills and experience more activation in their brain (Segelken, 2006). For years Haskins Laboratories, a nonprofit institution working with Yale, has researched dyslexia and other reading disorders. A more recent study, found differences in the brain between dyslexic children and normal readers while completing tasks relating to visuospatial processing. The study participants were hooked up to a fMRI to track their speed and precision and had to carry out tasks such as reading in print and processing figures. From the results, scientists learned that more brain stimulation occurred in the basal ganglia, an area of the brain responsible for learning, in non-dyslexic brains when processing the figure tasks than reading in print when compared to adolescents with dyslexia who experienced more activation in their brains when processing reading print. When conducting the specified tasks, average readers had greater activation in the left side of the brain while the dyslexics "lack the specialization for language processing in their left hemispheres [and] have less hemispherically differentiated activity"(Hayashi, 2014). This backs the idea of the hemispheric tradeoff hypothesis in which when performing one task the brain has limited room to multitask. Through this important research was able to disprove theory that dyslexia was correlated with a improper function of the human body's neurobiological system (Hayashi, 2014).

Current research using neuroplasticity, the process in which neurons establish new attachments between each other shows importance to the learning process in dyslexia. By conducting a functional MRI or fMRI, a scan of the human brain which shows a flow of blood to activated regions of the brain while the patient is performing certain tasks. Through the study of fMRI scans of dyslexics and non-dyslexics before and after remedial reading training scientists found that even after a year post training the activation in dyslexics their brain continued to the point that there were normal levels in the left parietal lobe. These results prove neuroplasticity of the brain. Not only did the remedial training increase brain activity, but it strengthened the brain's connections further improving the dyslexic children's ability to read. Nadine Gaab, conducted fMRI scans on 45 children, 22 of which had developmental dyslexia and 23 did not, all of these kids were 10 years old. Along with Gaab, colleagues at the Massachusetts Institute of Technology aided this investigation of dyslexia, the results showed that when the kids heard speech sounds the non-dyslexics experienced brain activity in their left frontal lobe while dyslexics did not. Gaab's data backs those of Paula Tallal from over 30 years ago when dyslexia was correlated to issues with processing speech sounds. In similar research conducted the Yale Center for the Study of Learning and Attention,

Sally Shaywitz the director took fMRI scans of dyslexic and average readers while reading nonsense words. According to the data, the study found increased brain stimulation located in the left hemisphere of the brain in the parieto-temporal region. Clearly, dyslexia does not cover just one area of the brain, but instead impacts many. Now with extensive research done in the field of dyslexia, hopefully new interventions and treatments will be available (Valeo,2008).

In consonance with professionals working alongside school age children who have dyslexia, sports can have an immense impact on the lives of dyslexics. The article, "Sports: Strengthening Their Self Confidence and School Skills" suggests that children should "guide the choice … but parents should help them to figure out what they're good at and what they enjoy. (Hall, 2016)" Evidently, kids should participate in sports that they find fun. From their experiences in playing sports these children will learn important lessons of acceptance, teamwork, concentration, and confidence. One dyslexic correlated rowing to writing a paper because "both require planning ahead and making sure you have all your equipment before a competition is like making sure you have all your research outlined before you start writing" the student derived that sports and academics both require time management and extensive focus. Clearly, physical activity is a useful intervention not only for your average child; it is far more effective for dyslexics (Hall, 2016).

With the aid of information and communications technologies (ICT), dyslexics can improve their education and learning skills. Recent research done by scientists in Greece conducted a study to identify whether adolescents with dyslexics test better on paper or digitally. The specified experiment used 20 students, 10 dyslexic and 10 non-dyslexic (control group), every child took both the computer and paper test on solving math problems. To avoid inconsistencies of accuracy the students were scrutinized by teachers while taking the computer based exam (Anestis, 2015). The data showed that all students had improved scores on the digital test compared to the paper one. However, the individuals with dyslexia had a 18 percent improvement on the electronic exam compared with the control who only had a small improvement of 9 percent (Anestis, 2015). As you can see, the use of ICT with adolescents with dyslexia is a better test taking method than the traditional paper test (Anestis, 2015).

Dyslexia can affect an individual's musical abilities. For instance, people with this neurological disorder may struggle with sight reading, remembering instructions, understanding terms and have difficulty with organization.. However, many interventions can be utilized to help improve the areas dyslexics struggle with. Always, it is important to have a teacher who understands the condition not only in music, but in all classes and teachers should be willing to go out of their way to enhance a student's learning experience. In addition, the teacher should provide alternative exams with a multisensory approach or exempt the student from these activities. Teachers should try new and innovative techniques, as these are usually most effective and creativity will be appreciated (The British Dyslexia Association, 2016). Through the use of an innovative Android multimedia app, special education preservice teachers can learn specific techniques in order to help teach dyslexics how to further improve their reading skills. The app uses background information to explain the teaching strategies and includes a videos of specialized dyslexic teachers demonstrating each learning tool. It is greatly effective for teaching children with dyslexia because it includes a multisensory approach. A pilot study was conducted with 30 university students receiving a degree in special education, all university students took the same intro course, a assessment was given both before and after the utilization of the app. Although, there were slight issues with the software, the pilot study showed that the pre-special education teachers had more knowledge after trying out the app. Not only improving the teacher's skills, the technology helped them better understand dyslexics on an emotional level (Osman, 2015).

A Swedish study of dyslexia in university students assessed dyslexic adults, in more than 8 different tests including RAN, reading comprehension, a teacher and student interviews. Based on previous research, adult dyslexics were able to cope with their reading issues by using context clues and background knowledge. Although, adults with dyslexia may read at average level "had verba developed a qualitatively different word decoding ability with a less specific orthographic knowledge and show difficulties in lower level decoding and spelling" (Olofsson, 2012). When testing the 95 participants, each individual session ranged from 1 hour 1.5 hours. The findings expressed that the students had low performance reading skills when using precision and timing. According to the participant questionnaire, most dyslexics struggled with note taking and reading in both Swedish and English. In addition, the participant interview gave verbal answers allowing for a more kinesthetic view of interventions and tools that helped dyslexics such as using audiobooks, working collaboratively, taking notes, reducing or shortening the text for reading and looking for summaries to article in order to read a short amount while still processing the most important information (Olofsson, 2012).

Dyslexics often struggle with reading fluency,reverse letters, and omit words. Additionally, they have issues tracking the words on the page while reading. As a result, many studies based off the visual elements of reading have been conducted on dyslexics. For instance, research based on font color while reading on a computer determined that text that is in dark letters on a light background is most readable for dyslexics. Specifically, this study showed that text is most

readable when the computer display is at a high brightness (Pijpker). Additionally, other studies found that simpler backgrounds are easier to read (LDAU). Therefore, combinations of darker text and a light backgrounds will speed up the rate at which the text is read (Pijpker). However, less research has been conducted relating to various font colors, backgrounds, the use of the finger for tracking, spacing, and tracking with a note card on printed paper with tests of eye tracking and reading fluency (Bloodsworth, 1992).

Goal of Study

The specified study will aim to analyze the implementation of specific visual elements for dyslexics while reading aloud. The research will determine if these adaptations will improve their fluency, assuage the frequency of skipping of lines and omitting words and will track eye movements.

Materials and Methods

The following study will use 40 children, 20 dyslexic (10 M, 10 F) and 20 "normal" readers as the control group (10 M, 10 F), all participants were included if they were 12-18 years of age.

Participants were excluded if they presented with with specific neurological issues such as Autism, Aspergers, Downs Syndrome and visual issues.

The mobile glasses light-pupil eye-tracker device (GET) manufactured by Tobii will be used to both record audio and video in order help analyze the pupils data when reading aloud with different visual elements. A mobile eye tracker will be used to allow subjects to move freely and read in natural environments ("What is eye tracking and how does it work?", 2016). The eye tracker device will measure the following:

Gaze points and fixations: Definitely the most prominent metrics in eye tracking literature. Gaze points constitute the basic unit of measure – one gaze point equals one raw sample captured by the eye tracker. Gaze points can be aggregated into fixations, a period in which our eyes are locked toward a specific object.

Areas of interest (AOI) and heat maps: To concentrate the analysis on specific regions on the stimulus, areas of interest (AOI) can be defined. Heatmaps visualize fixation positions and temporal changes of fixations as an overlay on a specific stimulus across different respondents.

Fixation sequences and further metrics: There are several ways to analyze gaze positions and fixations, for example you can analyze the fixation sequences and the performance of different regions in an image or a video with respect to the time of first fixation (TTFF) or the number of respondents looking toward a specified region (respondent count).

("What is eye tracking and how does it work?", 2016)

After each section, the eye tracker must be recalibrated to ensure complete accuracy. Also, following each session, the amount of time it takes the kids to read will be recorded for fluency. In addition, common reading errors made by dyslexics will be recorded (see chart 1). For each visual implementation the partipants will read one short paragraph aloud with a printed paper. During the midpoint of the full exersise aa 15 minute break will be administered.

Tracking Tasks

Method (with black size 12 arial font, normal spacing 1.15)
Finger tracking
Notecard tracking

Different background colors and fonts

Method (with size 12 arial font, normal spacing)	Background color	Text
Background color (control)	white	black
Background color	black	white

Different Spacing

Methods (with black size 12 arial font, white background)	Spacing number
Spacing (normal)	1.15
Spacing	1.5
Spacing	2.5

Different Font colors

Methods					
Font color	red	orange	green	blue	purple

Expected Results

Hypothesis A, Spacing: The spacing of 3.5 will be most effective in reducing errors because the further apart the text is from each other and therefore it is assumed that it will be harder to skip lines.

Hypothesis B, Text background: The normal white background with black font will the most effective for dyslexics because lighter backgrounds and darker fonts are easier to read.

Hypothesis C, Font color:The blue or red font will most effective for dyslexics while reading aloud.

Hypothesis D, Finger Tracking: Finger tracking will help

Overview reading errors

Reading error	*Description*
Switching letters	Switching of mirror letters like 'b' and 'd' or rotating letters like 'e' and 'a'.
Complex vowels	Complex vowels consists of two vowels. A complex vowel is replaced by another complex vowel or double vowel.
Open or closed syllables	An open syllable is read closed or a closed syllable is read open.
Consonant cluster shift or deletion	In a cluster of consonants some consonants are shifted in the word or deleted.
Deletion or addition of letters	A letter is deleted or added.
Deletion or addition of words	A word is deleted or added.
Replacement of words	A word is replaced by another word.
Read guessing	A word is guessed instead of well read.
Added reading errors	
Word reverse	A word is reversed with another word.
Number error	A number in the text is read wrong.
Pronunciation error	A letter is pronounced wrong, like 'c' pronounced as 'k'.

dyslexics read and reduce the frequency of skipping lines and losing track of their place.

Hypothesis E, Notecard Tracking:Note tracking will aid dyslexics while reading aloud and reduce the frequency of skipping lines and losing track of there place.

References

Anestis, E. (2015). The Effects of Using Information and Communication Technologies Instead of Traditional Paper Based Test, During the Examination Process, on Students with Dyslexia. Procedia Computer Science, 65, 168-175. http://dx.doi.org/10.1016/j.procs.2015.09.105

Belluck, P. (2016). Study Says Dyslexia May Affect Listening Comprehension. Nytimes.com. Retrieved 7 December 2016, from http://www.nytimes.com/2011/08/02/health/research/02dyslexia.html

British Dyslexia Association,. (2017). Music and Dyslexia. Bdadyslexia.org.uk. Retrieved 9 January 2017, from http://www.bdadyslexia.org.uk/educator/music-and-dyslexia

Dyslexia and the Brain: What Does Current Research Tell Us?. (2015). Ldonline.org. Retrieved 12 December 2016, from http://www.ldonline.org/article/14907

Early Levels of Representation in Reading , Johns Hopkins University. (2016). Sites.krieger.jhu.edu. Retrieved 13 December 2016, from http://sites.krieger.jhu.edu/mccloskey-lab/current-projects/early-levels-of-representation-in-reading/

Hall, N. (2016). Sports: Strengthening Their Self Confidence and School Skills. Dyslexia.yale.edu. Retrieved 5 December 2016, from http://dyslexia.yale.edu/article_print.php?a=PAR_SportsDyslexia

Hayashi, Y. (2014). Uncharted Waters in Dyslexia Research. Yale Scientific Magazine. Retrieved 13 December 2016, from http://www.yalescientific.org/2014/12/uncharted-waters-in-dyslexia-research/

Nasr, Susan. "Slow Reading In Dyslexia Tied To Disorganized Brain Tracts". Harvard Gazette. Nap. 2007. Web. 8 Dec. 2016 http://news.harvard.edu/gazette/story/2007/12/slow-reading-in-dyslexia-tied-to-disorganized-brain-tracts/

Norton, E., Black, J., Stanley, L., Tanaka, H., Gabrieli, J., Sawyer, C., & Hoeft, F. (2014). Functional neuroanatomical evidence for the double-deficit hypothesis of developmental dyslexia. Neuropsychologia, 61, 235–246. http://dx.doi.org/10.1016/j

Behe, B. K., Fernandez, T., Huddleston, P. T., Minahan, S., Getter, K. L., Sage, L., & Jones, A. M. (n.d.). Practical Field Use of Eye-tracking Devices for Consumer ... Retrieved May 23, 2017, from http://www.hrt.msu.edu/uploads/535/78629/ETT-Methods-Horttech.pd

Bloodsworth, J. G. (1992, November 30). Legibility of Print. Retrieved May 23, 2017, from https://eric.ed.gov/?id=ED355497

Desroches, A. S., Joanisse, M. F., & Robertson, E. K. (2006). Specific phonological impairments in dyslexia revealed by eyetracking. Cognition,100(3). doi:10.1016/j.cognition.2005.09.001

Diagnostic and statistical manual of mental disorders: DSM-IV. (2000). Index. (2012). The Handbook of the Neuropsychology of Language, 904-905. doi:10.1002/9781118432501.index

Norton, E. S., Black, J. M., Stanley, L. M., Tanaka, H., Gabrieli, J. D., Sawyer, C., & Hoeft, F. (2014). Functional neuroanatomical evidence for the double-deficit hypothesis of developmental dyslexia. Neuropsychologia,61, 235-246.Optimal Colors to Improve Readability for People with Dyslexia. (n.d.). Retrieved May 23, 2017, from https://www.w3.org/WAI/RD/2012/text-customization/r11

Pijpker, C. (n.d.). Reading Performance Of Dyslexics With A Special Font And A Colored Background - University Of Twente Student Theses. Retrieved May 23, 2017, from http://essay.utwente.nl/63321/

Tips to Make Your Text Easier to Read. (n.d.). Retrieved May 23, 2017, from http://www.ldau.org/ld-facts/guidelines-for-font-size-color/

Träff, U., Desoete, A., & Passolunghi, M. C. (2016). Symbolic and non-symbolic number processing in children with developmental dyslexia. Learning and Individual Differences. doi:10.1016/j.lindif.2016.10.010

What is eye tracking and how does it work? (2016, October 28). Retrieved May 23, 2017, from https://imotions.com/blog/eye-tracking-work/

Colony Collapse Disorder in Apiology, by Thea Barbelet (Sophomore)

Honey bee, Apis mellifera, populations around the world have been steadily decreasing, triggering public attention yet no solution (Dainat et al., 2009). In the United States, honey bees are considered to be one of the most important native pollinators by the Natural Resources Conservation Service (Marks et al., 2005). "Colony Collapse Disorder", a focus of many studies in the United States and Europe, has been treated as the main factor of these losses (Dainat et al., 2009). Characteristics of Colony Collapse Disorder, or CCD, include the departure of most worker bees in a hive, but not the queen or brood. This eventually results in failure of the hive, as it cannot be maintained (Cresswell et al., 2011). The widespread use of pesticides, which include neonicotinoids, a new type of nicotine-mimicking insecticide, fungicides, and herbicides are being researched as stressors of CCD (Hladik et al., 2016). Contributing factors of CCD also include the mite Varroa destructor, the single-celled gut parasite Nosema apis, and the parasite Nosema ceranae (Pettis et al., 2013).

Professional and public application of pesticides causes them to spread throughout an entire ecosystem, affecting surface water, groundwater, soil, turf, and the air (Aktar et al., 2009). Thiamethoxam, a likely human carcinogen used to coat seeds, and bifenthrin, a possible human carcinogen applied in aerosol or granule form, are the most commonly detected in captured wild bees from both grasslands and wheat fields in Colorado (Hladik et al., 2016). In 2014, thiamethoxam was found in half of all samples taken from wheat fields and about 47% of samples taken from grasslands at the same time (Hladik et al., 2016). Bifenthrin was detected in about 46% of all wheat field samples from 2014, and 30% of grassland samples (Hladik et al., 2016). Bees kept in apiaries show even more detections of residue. A combination of fluvalinate, which may cause birth defects, and coumaphos was detected in 83.1% of samples from a North American study (Mullin et al., 2010). Some pesticides containing coumaphos are restricted by the US Environmental Protection Agency due to its high fatality risk in humans While there are discrepancies, such as usage of different pesticides and

different methods of application, between bees in an agricultural setting compared to bees in a more urban setting, hives feel the effects everywhere.

Bees inadvertently bring these insecticides back into the hive, which then contaminates pollen, nectar, bee bread, jelly, and honey. Bees do have a detoxification system that can effectively modify dietary nicotine into harmless metabolites, but when placed under excessive stress, a loss of vitality occurs. Since there are bees of different ages and of different job classes, a correlation between the amount of labor and required energy to function arises. For example, foragers are more exposed to pesticides because they leave the hive, but have a diet far higher in protein than workers, and therefore can metabolize nicotine with less stress (du Rand et al., 2005).

Besides this limited detoxification system, bees have no natural resistance to insecticides nor pathogens they have previously not encountered (Goulson et al., 2015). Pesticide exposure leaves bees more susceptible to parasites such as Varroa destructor, Nosema apis, and Nosema ceranae which can then cause a multitude of devastating viruses, including acute bee paralysis virus, Israeli paralysis virus, Kashmir bee virus, and deformed wing virus. Diseases are easily spread throughout a weakened colony, and can even affect nearby hives (Sánchez-Bayo et al., 2016). Therefore, a colony somehow isolated from human chemicals can still be burdened by them.

Past studies are not without flaw. For example, Hladik reported that more in-depth studies are required to better explain the correlation between native bees and their behavior as well as their biology compared to pathogens. In the future, metabolism rates and foraging distances of specific species need to be examined, since bees may forage outside of their immediate area and become exposed to different pesticides than those found near the hive. Fungicides and herbicides are not as lethal but may have other side effects, which should be evaluated. Since only live specimens were taken, sublethal amounts of pesticides were accounted for, not how much it actually takes to kill a bee (Hladik et al., 2016). No studies have found the tolerance capacity regarding pesticides in a hive, or exactly how much insecticides bees may bring back into the hive (du Rand et al., 2015).

If this research becomes available, solutions may come with it. Since strictly outlawing all pesticides may never happen, researchers should work to strengthen the bees themselves. Decreased susceptibility to pesticides would be a direct result of increased bee immunity. Modifying a bee's detoxification system could also allow it to metabolize more neonicotinoids, making their application harmless. From a different standpoint, protecting a hive from foreign bodies would limit only the workers who foraged to pesticide exposure. Nectar, honey, pollen, and the worker bees themselves would all have to be sanitized. Miticides that kill only pathogens could be made, but may not be the best approach. Beekeepers such as Randy Oliver consider it best to wean colonies off of chemicals altogether, even organic or natural ones, and once genetic changes occur, in terms of immunity or strength, they can protect themselves.

References

Cresswell, J. E., Desneux, N., & vanEngelsdorp, D. (2012). Dietary traces of neonicotinoid pesticides as a cause of population declines in honey bees: An evaluation by Hill's epidemiological criteria. Pest Management Science, 68(6). https://doi.org/10.1002/ps.3290

Dainat, B., Evans, J. D., Chen, Y. P., Gauthier, L., & Neumann, P. (2012). Dead or alive: deformed wing virus and Varroa destructor reduce the life span of winter honeybees. Applied and Environmental Microbiology, 78(4), 981–7. https://doi.org/10.1128/AEM.06537-11

Dave Goulson, Elizabeth Nicholls, Cristina Botías, E. L. R. (2015). Bee declines driven by combined stress from parasites pesticides and lack of flowers. Sciencexpress.

Du Rand, E. E., Smit, S., Beukes, M., Apostolides, Z., Pirk, C. W. W., & Nicolson, S. W. (2015). Detoxification mechanisms of honey bees (Apis mellifera) resulting in tolerance of dietary nicotine. Nature Publishing Group. https://doi.org/10.1038/srep11779

Habitat, W., & Leaflet, M. (2005). Native Pollinators, (34), 1–10.

Hladik, M. L., Vandever, M., & Smalling, K. L. (2016). Exposure of native bees foraging in an agricultural landscape to current-use pesticides. Science of the Total Environment. https://doi.org/10.1016/j.scitotenv.2015.10.077

Pettis, J. S., Lichtenberg, E. M., Andree, M., Stitzinger, J., Rose, R., vanEngelsdorp, D., … Pham-Delegue, M. (2013). Crop Pollination Exposes Honey Bees to Pesticides Which Alters Their Susceptibility to the Gut Pathogen Nosema ceranae. PLoS ONE, 8(7), e70182. https://doi.org/10.1371/journal.pone.0070182

Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., & Desneux, N. (2016). Are bee diseases linked to pesticides? - A brief review. Environment International. https://doi.org/10.1016/j.envint.2016.01.009

A Study on Single-domain Antibodies by Buu-Hac Nguyen (Sophomore)

Single-domain Antibodies (sdAb), also known as nanobodies, are antibody fragments that are derived from camelids (Cortez-Retamozo et al., 2004). Single-domain antibodies are used from camelids because the antibodies are comprised of a heavy chain homodimer rather than of light chains (Saerens et al., 2010). Studies have shown the antigen-binding portion of the heavy-chain antibodies, which constitutes the complete nanobody, has a greater tendency to interact with parts of the target that are not easily recognized by conventional antibodies because of their smaller size, which allows access to hard-to-reach areas of the target (Saerens et al., 2010). Although these nanobodies are free from light chains, the nanobodies remain fully functional (Cortez-Retamozo et al., 2004). They are stable, highly soluble, distinctly specific and have high affinity (Cortez-Retamozo et

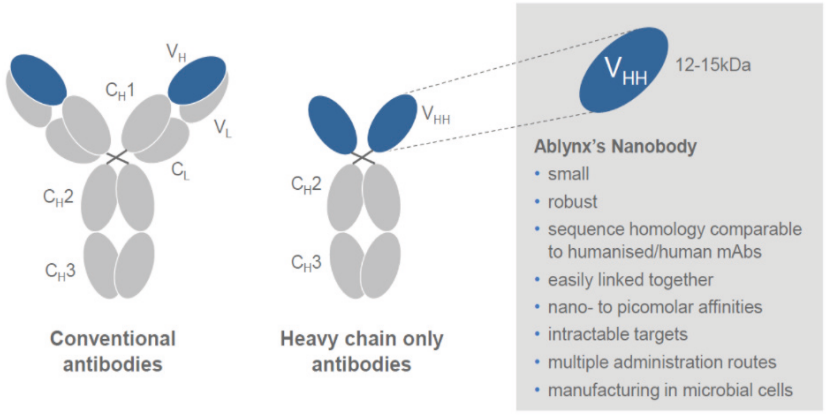
al., 2004).

Through these favorable characteristics and their minuscule size, scientists are examining the potential uses of single-domain antibodies in biosensing applications as well as treating diseases through isolating, cloning, and selecting nanobodies with specificity to the desired antigens (Cortez-Retamozo et al., 2004). The results of these studies exhibit the possible use of nanobodies in the dopamine transporter, a protein that forces the neurotransmitter dopamine into presynaptic neurons.

To determine the potential use of nanobodies in biosensing and diagnostic assays, scientists secured single-domain antibodies onto biosensor surfaces with the use of multiple immobilization strategies (Pia et al., 2015). The characterization of the interaction with the single-domain antibodies' specific target was done by Surface Plasmon Resonance (SPR) biosensors, which are used to monitor biomolecular interactions due to their high sensitivity and reproducibility (Pia et al., 2015). Through the immobilization methods, scientists determined the kinetic binding constants of the immobilized nanobodies for their antigens on the different surfaces. This data was compared to traditional monoclonal antibodies with their kinetic binding constants and showed that the immobilized nanobodies were the most successful in capturing molecules (Pia et al., 2015). Furthermore, the results of this study also revealed immobilized nanobodies with high affinities to their antigens and high resistance to numerous denaturing agents, such as temperature. These characteristics portray the possibility of using nanobodies in the dopamine transporter because the nanobody is likely to identify the dopamine transporter without becoming impaired.

In addition, nanobodies have the ability to treat diseases, such as cancer, by attaching to the tumor site and killing cancer cells (McMurphy et al., 2014). Previous studies have shown that nanobodies specific to carcinoembryonic antigen (CEA), a protein which appears in the blood of cancer patients, targeted a distinct non-overlapping epitope on the CEA molecule (Cortez-Retamozo et al., 2004). An experiment revealed how the nanobody conjugate stopped the growth of the tumor xenograft that was placed in nude mice (Cortez-Retamozo et al., 2004). These results show that the minuscule size of single-domain antibodies alters their biodistribution and distinctly improves access to epitopes (Cortez-Retamozo et al., 2004). The improved access to epitopes further illustrates the likelihood of using nanobodies in the dopamine transporter because of their smaller size, as compared to whole antibodies, which has better access to the dopamine transporter.

Furthermore, a previous study shows the application of specific whole antibodies in the dopamine transporter (DAT) (Ciliax et al., 1995). The antibodies were used for immunolocalization of transporter protein in rat brain (Ciliax et al., 1995). This study showed how the antibodies targeting the N-terminus and the C-terminus were specific to the expressed cloned DAT, recognized the transporter protein and were sensitive to the excess homologous fusion protein (Ciliax et al., 1995). The results of this study prove how the generation of specific DAT antibodies will allow further characterization of the cellular as well as subcellular localization of DAT protein (Ciliax et al., 1995).



Mentor: Dr. Matthias Quick, Ph.D.

ASSISTANT PROFESSOR of Clinical Neurobiology, Department of Psychiatry and Center for Molecular Recognition,

Columbia University and College of Physicians and Surgeons.

We are very grateful for the dedication and help of Dr. Quick.

Compared to whole antibodies, nanobodies are significantly smaller, which alters the biodistribution of the single-domain antibodies and improves access to the target. Nanobodies also show high affinities for their target and remain stable under denaturing agents; with these superior characteristics, nanobodies portray potential use on the dopamine transporter. The use of nanobodies on the dopamine transporter exhibit promising advancements in immunotherapy.

References

Cortez-Retamozo et al, (2004), Cancer Research, 4, n.a., 2853–2857, Efficient Cancer Therapy with a Nanobody-Based Conjugate.

Della Pia, E. A. & Martinez, K. L., (2015), PLOS ONE, 10, n.a., 1-17. Single Domain Antibodies as a Powerful Tool for High Quality Surface Plasmon Resonance Studies.

McMurphy et al, (2014), PLOS ONE, 9, n.a., 1-8. The Anti-Tumor Activity of a Neutralizing Nanobody Targeting Leptin Receptor in a Mouse Model of Melanoma.

Saerens, D., (2010), World journal of biological chemistry, 1, n.a., 235-238. Isolation and optimization of camelid single-domain antibodies: Dirk Saerens' work on Nanobodies.

Vaughan, R. A., & Foster, J. D. (2013). Mechanisms of dopamine transporter regulation in normal and disease states. Trends in Pharmacological Sciences, 34(9)

Ciliax et al, (1995), The Journal of Neuroscience, 75(3): 1714-1723. The Dopamine Transporter: Immunochemical Characterization and Localization in Brain.

Ablynx's Nanobody [Digital image]. (2015, August 11). Retrieved Dec. & jan., 16, from http://seekingalpha.com/article/3429536-ablynx-unlike-any-other-biotech-company

Developmental Dyslexia by Jason Uyaguari (Sophomore)

The study of dyslexia is very important to a lot of people. Developmental dyslexia is affecting about seven percent of school age children (Peterson,2012). So advancements in the understanding of dyslexia, Would benefit a lot of people.There are many questions still at hand when it comes to dyslexia. I want to answer a couple of those key questions. Is there a correlation between a poor understanding of your own languages phonology and more severe effects of dyslexia? If so how can we use this to help people with Dyslexia? Phonology is described as the relationship between certain speech sounds that make up important parts of a language.

In many studies the phonological factor usually has a significant impact on overall ability in the tests. If you look at the neuropsychologia (Hoeft,2010) research paper. Then you will see how the subject's scores in the phonology tests correlate with their other ones. Also in the the remediations paper and implications with dyslexia (kershner,2012). They speak of a remediation plan called interactive specialization. Where through repeated phonological exercises they form reciprocal pathways in the brain. There are many studies that link phonology as a factor if not a main cause of dyslexia.Through correlations and other data their relationship becomes more and more clear.

Children with dyslexia have a different neuroanatomical makeup in their brains compared to people without it.Which basically means the structure in their brain is different than a control's brain. In this study they took a control group of people and looked at their MRI's and and looked at their right hemisphere. You can see differences in concentration of white and grey matter in different parts of it such as the corpus gyrus.The difference in anatomy is shown to be significant. (Cutini,2016).

New research has led scientists believe that a new side effect of dyslexia is sensory and motor deficits called sensorimotor syndrome. This has led to two theories in the research of dyslexia . One that the the reading impairment is rooted in the left peri-sylvian origin and that it is primarily caused by the phonological problems in the brain. The Alternate theory is about the motor and auditory deficits that lead to phonological deficits and then the rest is very similar in both theories. (Ramus, 2003).

Also found in a foundationally unique study. Subjects from three different cultures were taken for this study on dyslexia. When given different reading tasks and under a PET scan they show reduced activation in parts of the brain that deal with reading (left temporal and occipital lobes). They show this as a result of different amounts of concentration of grey and white matter. This supports the theory that dyslexia is connected to the phonological and other reading parts of the brain. Explicitly demonstrating that the anatomical makeup, In someone with developmental dyslexia (Silani, 2005).

People with dyslexia develop differently than people in a large study was conducted with children with developmental dyslexia in a number of tests with phonological tests and and reading tests.

With four different subgroups (DYS older) (DYS younger), (TD younger)and (TD older). As expected the results differed but the similarity between The (DYS older) and (TD younger) was unusual. They also had different concentrations of white and grey matter in their brains ,with the (DYS younger) and)DYS older) (Hoeft, 2010).

Scientists Agree there's a phonological factor to dyslexia, and they want to compare people with dyslexia to their age matched controls in a series of tests from counting syllables to rhyming words. It's determined that they have

deficits in performing tasks like this . With the phonology of their languages. Also found is that training the phonological aspect of it improves visual dyslexia and helps with their reading and writing scores in their tasks (Colling 2016).

Dyslexic readers react differently to different number of oscillations (Hz) ex: there is a negative correlation in phonological skills when alpha oscillations are used contrastingly there is a positive correlation when beta oscillations are used. This is one of the more clearer examples of the effects phonology has on this condition.(De vos,2016)

But there's also been another more overlooked aspect of dyslexia, which is juggling multitasking in the brain. In a study 23 teens (age 13) were taken with their age matched controls were asked to perform single tasks like counting backwards. But when asked to do a dual task paradigm (simultaneously doing tasks). 19 of the 23 dyslexic kids showed a lot of impairment doing the tasks.This exposes the intricacy of dyslexia and how it makes even how we foundationally think different (Fawcett ,1990).

However There has been research in methods to help with the deficits of dyslexia. there appear to be some fractional ways to help dyslexic people. This paper offers a phonological approach to it called interactive specialisation. Under the system they try to make new pathways of reciprocal competitive areas. This has been proved to help dyslexic readers with core problems in testing (Kershner, 2016).

If someone gave me a chance to research developmental dyslexia With funding.I would try to determine the extent of the phonoligical factor in dyslexia.How much does it effect the reading and writing? How does their anatomical makeup differ from people in the way that, people use the parts of the brain differently than variables? And most importantly How much can be remediated through Phonological treatment such as interactive specialisation. But there is still a lot more work to be done on research in dyslexia .

References

De vos, Atypical neural synchronization to speech envelope modulations in dyslexia ,(2017), Brain and language, 164, 106-117, http://dx.doi.org/10.1016/j.bandl.2016.10.002)

Cutini, S., Szűcs, D., Mead, N., Huss, M., & Goswami, U. (2016, December). Atypical right hemisphere response to slow temporal modulations in children with developmental dyslexia. Retrieved April 25, 2017, from https://www.ncbi.nlm.nih.gov/pubmed/27520749

4 Neural encoding of the speech envelope by children with developmental dyslexia (power, Brain and Language,2016 Centre for Neuroscience in Education,http://www.sciencedirect.com/science/article/pii/S0093934X15301681)

5 Shared temporoparietal dysfunction in dyslexia and typical readers with discrepancy in High IQ (Hoeft ,trends in neuroscience,2016 volume 5 issue 4 http://www.sciencedirect.com/science/article/pii/S2211949316300503)

6 .Review and implications for remediation(Walker,The Neurophysiology of Dyslexia,2004,pages 45-55 http://www.tandfonline.com/doi/abs/10.1300/J184v10n01_04)

7 Automaticity a new framework for dyslexia research (Cognition.Fawcett.1990.volume 35 issue 2 http://www.sciencedirect.com/science/article/pii/001002779090013A)

8 Developmental dyslexia specific phonological deficit or general sensorimotor dysfunctio(Ramus,Neurobiology,2003, volume8 https://www.ncbi.nlm.nih.gov/pubmed/12744976)

9 .Brain abnormalities underlying altered activation in dyslexia: a voxel based morphometry study (Silani, VBM study,2005 volume 10,https://academic.oup.com/brain/article-lookup/doi/10.1093/brain/awh 579)

10 The evidence for a temporal processing deficit linked to dyslexia (Farmer, M.E. & Klein, R.M. Psychon Bull Rev (1995) 2: 460. doi:10.3758/BF03210983)

Acknowledgments

This program would not be possible without:

Mentors and Scientists.
Thank you to all of the scientists who have taken our high school students under their wings and given them unprecedented possibilities to access genuine scientific research, to act as professionals and to express their talents and creativity in their projects.

- **Dr. Matthias Quick**, Ph.D. Assistant Professor, Department of Psychiatry and Center for Molecular Recognition, Columbia University, College of Physicians and Surgeons. Dr. Quick served on our Institutional Review Board and guided other science research students through their projects throughout the years. Dr. Quick served a judge in all the major competitions of the tri-county area, hereby gaining important knowledge of the competition proces.
- **Dr. Zhong Pan,** Ph.D. Post Doctoral Associate, New Jersey Institute of Technology, for welcoming Isabella Brizzi in his lab.
- **Dr. K.V. Lakshmi,** Ph.D. Associate Professor Chemistry and Chemical Biology. Scientific Director of the Baruch '60 Center for Biochemical Solar Energy Research . Rensselaer Polytechnic Institute. Troy, NY. We are grateful to Dr. Lakshmi for her dedication and help.
- **Dr. Emma Guttman,** Ph.D. Mount Sinai Medical School, ICahn Medical Institute. Professor Dermatology; Assoicate Professor Medicine, Clinical Immunology. Mount Sinai Medical School, ICahn Medical Institute. We are grateful to Dr. Guttman for her
- **Dr. Adam Zeman**, MD, Professor of Cognitive andBehavioral Neurology. University of Exeter, Medical School, Exeter, UK.We are very grateful to Dr. Zeman for his dedication and help
- **Dr. Stuart A. Newman,** Ph.D. Professor of Cell Biology and Anatomy. New York Medical College, Valhalla, NY. We are grateful to Dr. Newman for
- **Dr. Patric K. Stanton,** Ph.D. Professor of Cell Biology and Anatomy, New York Medical College, Valhalla, NY. We are grateful to Dr. Stanton for his help.
- **Dr. Ryan Baker, Ph.D.**, Associate Professor of Cognitive Studies in Education. Teachers College, Columbia University, NY

Sponsors:
We want to thank the Hendrick Hudson Community Educational Foundation (HHCEF) for its on-going funding of the science research program.

Parents
This program would not be successful without the support of the students' families, who sacrifice so much of their time to ensure that the students get the best possible experience when they work on site, in labs and during the school year.

Administrators, Teachers and Staff.

- **Mr. Joseph Hochreiter,** Superintendent
- **Dr. Margaret Ruller**, Executive Director of Curriculum and Instruction.
- **Mr. Enrique Catalan**, Assistant Superintendent for Business.
- **Mr. James Mackin** Principal.
- **Mrs. Tina Wilson**, Assistant Principal.
- **Mrs. Laura Neier,** Assistant Principal.
- **Dr. Mathew Swerdloff,** Executive Director of Educational Support Services.
- **Ms. Beth Gruber**, HHCEF.
- **Ms. Laurie Small** and **Ms. Jill Wheeler** for their sustaining support.
- **Ms. Clare Carey** for catering services
- **The custodial staff.**

Science Research Colleagues:

- **Mr. Michael Blueglass,** Yorktown High School
- **Mr. Angelo Piccirillo**, Ossining High School
- **Mrs.Valerie Holmes**, Ossining High School.
- **Mrs. Donna Light Donovan**, Croton-on-Hudson.
- **Mr. Leonard Behr and Dr. Daniel Wulff**, Science Research in the High School Program, University at Albany.

Members of the School Institutional Review Board:

Thank you for taking the time to review the work of students' projects througout the year.

- **Mr. James Mackin**, Principal.
- **Mrs. Tina Wilson,** Assistant Principal
- **Mrs. Ann-Marie Gallagher**, Science Teacher.
- **Mrs. Eileen Cancro**, Science Teacher
- **Mrs. Kathryn Frost,** Nurse.
- **Dr. Matthias Quick**, Assistant Professor of Clinical Neurobiology

REGENERON WESEF

Westchester Science & Engineering Fair

Entergy

ACORDA THERAPEUTICS

PsychoGenics

ZEISS

Westchester Academy of Medicine

FUJIFILM

PHILIPS

MOMENTIVE

TEATOWN LAKE RESERVATION
A Nature Preserve & Education Center

Westchester County Psychological Association

M&T Bank

NY BIOHUD VALLEY

The Clayton Family, Four Winds Hospital

Robert Weireter, Mianus River Gorge, Scenic Hudson, Omnico Promotions

For more information on the Science Research Program,
Contact Dr. Christine Rogers, Ph.D.
christine.rogers@henhudschools.org