



Hendrick Hudson
High School

Science

May 2014

Research

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“ You are limited only by your imagination”



Dr, Christine Rogers received a 2014 INTEL STS Badge and a Teacher of Merit Award.



Audrey Hecht is a semi-finalist for INTEL STS



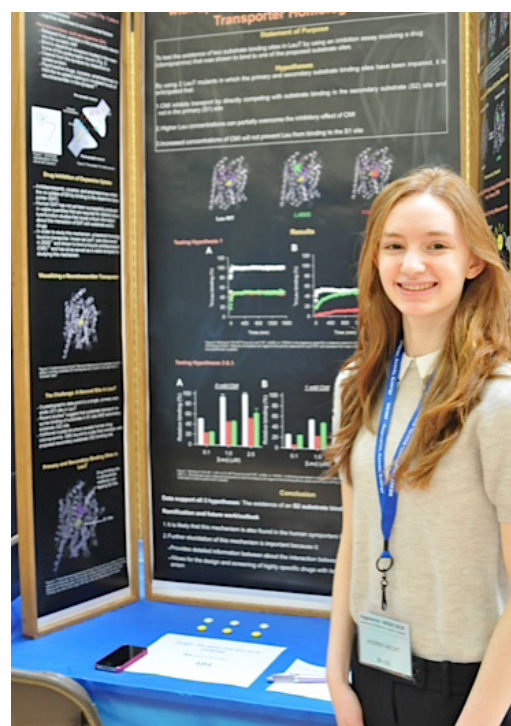
Dylan Gambardella received a badge for his entry to the INTEL STS competition.

Hendrick Hudson High School

Science *May 2014* Research

This year has been an amazing year for the Science Research Program. Audrey Hecht was one of the INTEL Science Talent Search semi-finalist for her excellent paper on the Dopamine Transporter. The INTEL STS is one of the most prestigious competitions and is paper-based. She placed 5th place in the Junior Science and Humanities Symposium (JSHS) and 4th Place at the Westchester Science and Engineering Fair (WESEF). Audrey has done a fantastic work under the guidance of her mentor Dr. Matthias Quick at Columbia University.

Dylan Gambardella won First Place at JSHS in the speaker category, allowing him to go to the JSHS New York State finals. He placed 2nd in the environmental science category at WESEF, allowing him to proceed to the I-SWEEEP competition (International sustainable world energy, engineering, environment project olympiad), a renowned international competition with over 66 countries and 385 projects competing. In Houston, he won the Gold Medal at I-SWEEEP in his cat-



egory. Dylan obtained First Place at the Tri-County competition in Environmental Sciences and will compete at the New York State level end of May. Dylan worked under the direction of Dr. Lung-Chi Chen at the New York University Langone Medical Center, showing how air pollution may trigger type 2 diabetes.

Both students did amazing work and will be continuing their research once in college. They have established many business and scientific connections useful for their professional achievements and have acquired invaluable skills which will allow them to be successful in future studies. Many Science Research Program alumni have continued their work once in college and have benefited from this outstanding experience.

Evaluating the Potential and Mechanisms of Diabetes Mellitus Induction in Response to Acute Ambient Particulate Pollution Exposure, by Dylan Gambardella. INTEL STS Paper excerpt.

ABSTRACT

This study explores the potential pulmonary and cardiometabolic effects of Fine Particulate Matter (PM_{2.5}) on a mouse model. More specifically, this research investigates pulmonary, diabetic (glucose levels, aortic rings, and insulin responses), and genetic factors. Studies have shown that higher levels of PM_{2.5} are linked with increased Diabetes prevalence, although experts have yet to clearly identify the underlying cause. Possible epigenetic/genetic modifications may occur or result from PM_{2.5} exposure and may be a possible link between air pollution and Diabetes. This experiment involves the use of an FVB/NJ inbred mouse model, with acute PM exposures taking place twice weekly for a total of three weeks. Mice were divided into three groups for the purpose of this experiment.

Two groups were exposed to PM_{2.5}, urban (Manhattan/Bronx NY) and rural (Goshen/Tuxedo/Wallkill NY) samplings, respectively, while the third served as the control (exposed to water). The objective of this experiment was to examine the induction of pulmonary, metabolic, and related genetic adverse health effects (genes related to and associated with insulin resistance) attributed to acute exposures to PM_{2.5} for the 22-day period. Researchers determined, using a RT² Profiler™ PCR Array Mouse Insulin Resistance, that certain genes are expressed through urban and rural pollution exposures by comparing the genetic results of the groups of mice simultaneously and analyzing for variations in gene expression. Differences in gene markers were noted and will be further investigated in future research. Pulmonary

and metabolic changes also demonstrated the adversarial effects of PM_{2.5} exposure.

The specific aims of this proposed study are to:

1. Assess pulmonary inflammation in response to exposure to urban and rural air pollution.
2. Assess metabolic perturbation indicative of overt diabetes in response to exposure to air pollution.
3. Assess if exposure to air pollution alters the expression of insulin resistance related genes in mice.

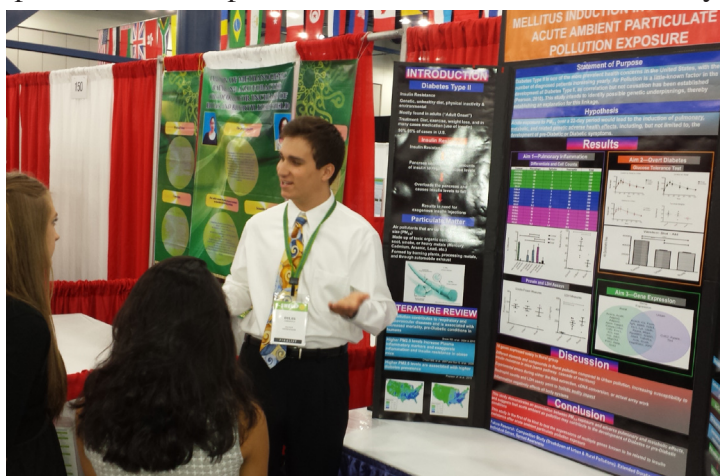
INTRODUCTION and RESEARCH STRATEGY

Significance

The proposed study is relevant to one of the most prevalent health hazards. Each year, more than twenty five million Americans (approximately one out of every twelve people) are directly affected by Diabetes Mellitus (American Diabetes Association, 2011). Diabetes is a set of diseases, identified by high blood glucose levels, which result from the body's inability to produce and/or effectively use insulin. The disease can be broken down into two main groups: Type I and Type II. These two

variations of the disease differ greatly, each having distinct characteristics despite similar phenotypes (i.e. lethargy, increased urination, excess thirst, weight loss, blurred vision, frequent infections and slow wound healing), such that they are almost not the same disease (American Diabetes Association, 2011). For the purposes of this study, we will be focusing on Type II Diabetes, as the autoimmune components of Type I exclude exogenous particulate induction as a likely factor in development.

Diabetes Type II is often called "Adult Onset Diabetes," as it is mainly found in grownups. This type of Diabetes is known as "insulin resistant," and can be caused by genetic factors (as in Type I) and by inadequate insulin secretion from the pancreas due to beta cell dysfunction. Insulin resistance is attributed to elevated levels of free fatty acids in plasma, and often leads to decreased rates of glucose transport into muscle cells. This allows glucose to remain in the blood to a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues (American Diabe-



tes Association, 2004). Insulin resistance can also cause hepatic glucose production to elevate and an increased breakdown of fat (Khar-dori, 2011). In addition to predisposed genetic factors cited throughout the literature, this type of Diabetes can also be attributed to exogenous and environmental factors, such as an unhealthy diet, a lack of physical activity, and surprisingly pollution from the environment (American Diabetes Association, 2004). Type II accounts for the majority of Diabetes incidence worldwide and over ninety percent of Diabetes cases in the United States (American Diabetes Association, 2011).

Air pollution is a known but emerging factor in the prevalence of Type II Diabetes, as correlation but not causation between the two has been established (Pearson, 2010). A specific type of air pollution, fine particulate matter, is considered to be especially dangerous to humans. Fine particulate matter (PM) can be made up of toxic organic compounds, dust, dirt, soot, smoke, or heavy metals. PM is formed by burning plants, processing metals, and through automobile exhaust. Dr. W.E. Wilson describes the formation of particulate matter in detail: “The particle for-

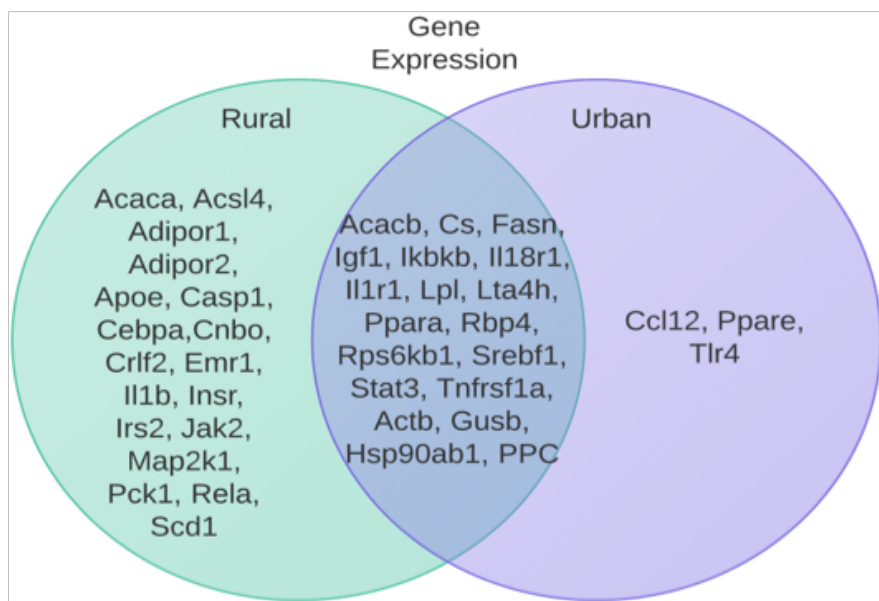
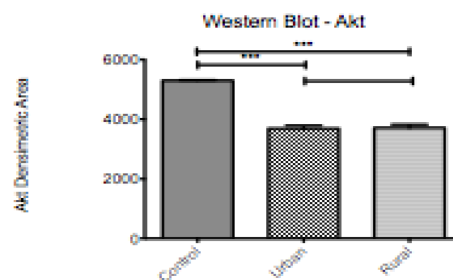
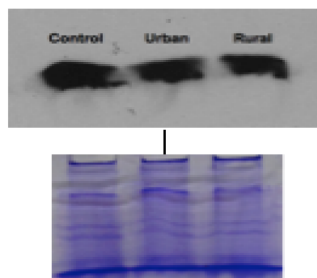
mation process includes nucleation of particles from low-vapor pressure gases emitted from sources or formed in the atmosphere, condensation of low-vapor pressure gasses on existing particles, and coagulation of particles,” (Wilson, 2002).

PM_{2.5} categorizes all particulate matter pollutants under 2.5 μ m, and is considered to be particularly harmful (Environmental Protection Agency, 2010). In a study conducted by MS O’Neill in 2006, long-term exposure to PM_{2.5} was found to be associated with a higher risk of relative mortality among people with diabetes, when compared with the general population. Research from 2010 showed that ambient fine particulate matter was found to induce insulin resistance in mice, causing symptoms similar to Type II Diabetes (Sun, 2010). The Environmental

Protection Agency credits the great health risks posed by PM_{2.5} to its ability to lodge deep within one’s lungs and circulatory system. This is due to its minute width (approximately 1/30 the average width of a human hair).

Air pollution has long been considered detrimental to one’s overall health (Brook, 2004). Recent studies have shown the effects of particulate matter on various bodily functions. In 2004, it was concluded, using data provided from the American Heart Association, that short-term exposure to PM led to an increased number of hospital admissions and cardiovascular mortality (Brook, 2004).

In addition, PM exposure has been observed to alter the platelet activation process in humans, leading to homeostatic irregularities (Cozzi, 2007). Fine particulate matter particles can also reach the circulatory system, causing obstructions and effecting blood flow (O’Neill, 2006). This type of blockage can lead to the formation of blood clots, which are known to be the leading cause of strokes (American Stroke Association, 2012). Fine particulate matter is capable of interfering with the lungs, as well, by depositing particles in the lower airways (gas exchanging portions of the lung), effectively disrupting the respiratory system and lead-



ing to numerous breathing problems (O'Neill, 2006). Though scientific research has demonstrated a relationship between Diabetes Mellitus and various components of air pollution, experts have yet to find the underlying cause. Transcriptional and post-transcriptional genetic influences (i.e. epigenetic changes) could possibly be feasible biological mechanisms that underlie the association between Diabetes prevalence and air pollution. Exposure to particulate matter may affect one's epigenetic makeup, conceivably modifying gene regulation, subsequently leading to the expression of possibly destructive genes, resulting in health concerns such as Diabetes.

This study explores the pulmonary, metabolic, and genetic impacts of fine particulate matter on patients using a mouse model. (...)

DISCUSSION and CONCLUSIONS

Findings demonstrate an association between PM2.5 exposure and adverse pulmonary and metabolic effects, suggesting that acute ambient air pollution may contribute to the development of Diabetes or pre-diabetic conditions, especially in urban environments. The outcomes from the pulmonary and metabolic aspects of this study are consistent with previously

completed experiments. Advances in technology, specifically the creation of the PCR Array, have allowed for insight into the underlying genetic link between air pollution and Diabetes. To the knowledge of this writer, this study is the first of its kind to test for the expression of multiple genes known to be related to insulin resistance as a result of acute ambient particulate pollution exposure. In addition, this study is the first to compare the expression results of mice exposed to urban pollutants with those of mice exposed to rural pollutants.

The results of the RT² Profiler™ Insulin Resistance PCR Array showed that 18 genes were expressed only in the group of mice exposed to rural pollution. It had been expected that certain genes might only be expressed in the urban group (which was the case for 3 genes), as the pollution samples from Manhattan and the Bronx are known to contain higher concentrations of PM2.5 than the rural pollution sampling, however this researcher did not anticipate multiple genes being expressed exclusively in the rural grouping. The high number of genes solely expressed in the rural group can stem from two possible causes:

1) The rural pollution (Goshen, Tuxedo, and Wallkill) is comprised of

certain elements and materials that are lacking in the urban pollution, and these components increase susceptibility to insulin resistance in mice.

2) Mechanical errors occurred during either the RNA extraction, cDNA conversion, or actual array work.

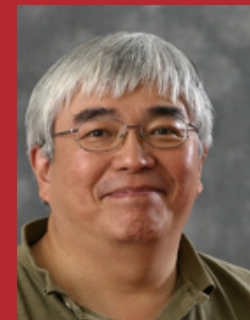
To confirm these findings, future researchers may wish to run the exposures and array work again, or create a study re-

searching particle composition. Such a study would require measuring the amounts of all elements present in pollution samples.

While the PCR array results, coupled with the pulmonary and metabolic data, indicate that exposure to ambient urban PM2.5 particles can result in unfavorable bodily impacts, the data also suggests that exposure to ambient rural PM2.5 particles can have adversarial health effects, as well. Considerable levels of neutrophils existed within the BAL fluid of the mice in this group, and LDH measures were not ideal, either.

Given the limited time frame of this study, certain known side effects of Diabetes, including weight gain, may not have had

Mentor: Dr. Lung-Chi Chen, Ph.D.



Professor, Department of Environmental Medicine. New York University Langone Medical Center. We are grateful to Dr. Chen for his guidance.

enough time to fully develop to the point of quantification. A similar study with an extended duration would help to determine if the results that are suggestive of the development of Diabetes or pre-diabetic conditions through prolonged exposure are properly labeled as such.

In addition, taking a closer look at the genes that were expressed in the PCR array by targeting each individually would provide more information concerning their roles in the induction of Diabetes Mellitus. Experimental timeframe may play a role in gene expression profile variation, thus limiting any potential temporal variances and should be considered as well in future studies.

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Interaction of a Tricyclic Antidepressant with Specific Sites in the Neurotransmitter Transporter Homolog LeuT, INTEL STS Semi-Finalist Paper excerpt, by Audrey Hecht

Abstract

Today, over 20 million Americans use antidepressants. Therapeutic antidepressants such as the tricyclic antidepressant (TCA) clomipramine (CMI) are psychoactive drugs that inhibit neurotransmitter reuptake from the synapse back into the presynaptic neuron. These drugs specifically target neurotransmitter: sodium symporter (NSS) proteins such as the transporters for dopamine, serotonin, and norepinephrine (DAT, SERT, and NET, respectively)

which use the Na⁺ and Cl⁻ electrochemical gradient to power the transport of their respective substrate into the presynaptic neuron. Using the bacterial NSS homolog LeuT from *Aquifex aeolicus*, crystallization studies demonstrated tricyclic antidepressants bound to a specific site in the protein called the extracellular vestibule located farther away from the substrate binding site (termed the primary or S1 binding site). Since the drug binding site does not overlap

with the S1 site, these data directly argue against a competitive drug inhibition mechanism. A recent study (Shi et al., 2008) concluded that in addition to the centrally-located S1 binding site there is a second substrate (S2) binding site that directly overlaps with the drug binding site in the extracellular vestibule. It is proposed the mechanism of TCA inhibition is based on competition of TCA with substrate binding to the S2 site.

Introduction

The interactions between nerve cells or neurons in the brain play a central role in human behavior as they control emotions and cognitive responses to environmental factors. Sensory neurons transmit information from receptors located throughout the body that detect external stimuli such as smells, sights, sounds, textures and taste, as well as internal stimuli such as muscle tension, carbon dioxide levels, and blood pressure. Received by dendrites, the

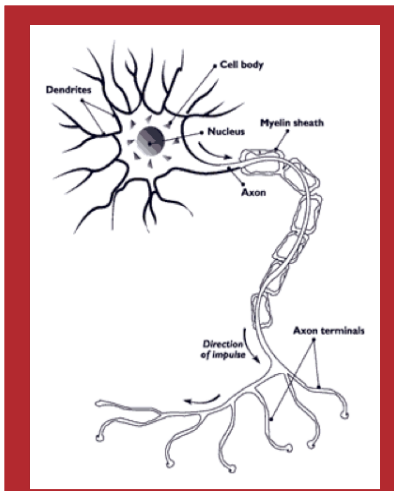


Figure 1. Schematic representation of the nerve cell (neuron).

branched projections on one end of the neuronal cell, these stimuli ‘travel’ along specialized neuron extensions, called axons, as electrochemical signals, i.e., action potentials, throughout the central nervous system. Near the

end of the axon, the neuron splits into several branches, called the axon terminals. At the extreme end of each axon terminal is the presynaptic terminal (Fig. 1). Together with the postsynaptic terminal located at the end of a dendrite of another neuron, these structures build the so-called synapse (Fig. 2). At the synapse, nerve impulses are carried from one neuron to another by means of chemical messengers called neurotransmitters. These neurotransmitters pass signals between neurons in the brain, thereby forming networks that regulate emotion and behavior

(Campbell & Reece, 2005).

Upon the release of neurotransmitters from synaptic vesicles located in the presynaptic terminals of the transmitting cell into the synapse, they bind to receptors on the postsynaptic membrane, located on the receiving cell (see Fig. 2 for a schematic representation). Binding of the neurotransmitter to the receptors leads to a cascade of events at the postsynaptic nerve ending that is responsible for the propagation of a nerve impulse. This sequence of molecular events is terminated by the reuptake of the neurotransmitter back into the presynaptic terminal, where it is recycled back into the communicating cell, thereby preventing the continued interaction of the neurotransmitter with the postsynaptic receptors. Different neurotransmitters, such as the monoamines (dopamine, serotonin, or norepinephrine) bind to specific receptors (Campbell & Reece, 2005). For example, the neurotransmission of dopamine (DA) influences behavioral, interactive, cognitive, physical, and emotional reactions. Dopaminergic neurotransmission ends with the removal of the neurotransmitter dopamine from the synapse by the reuptake of dopamine by the dopamine transporter, DAT. DAT together with other transport-

ers such as SERT, NET, and GAT (transporters for serotonin, norepinephrine, and GABA, respectively) belong to the SLC6 gene family, encompassing the human members of the neurotransmitter:sodium symporter (NSS) family (Chen et al., 2004). These symporters use the electrochemical sodium ion (Na⁺) gradient across the presynaptic membrane as source of energy to transport their respective substrate into the cell (Campbell & Reece, 2005).

Structure-function relationship of SLC6 transporters

The human transporters that mediate the presynaptic reuptake of monoamine neurotransmitters such as dopamine, serotonin and norepinephrine belong to the SLC6 gene family, a subgroup of the neurotransmitter:sodium symporter (NSS) family. SLC6 proteins regulate the levels of extracellular concentrations outside of the cell by mediating the reuptake of neurotransmitters across the cell membrane of the presynaptic neuron (Fig. 2). The SLC6 transporters mediate the co-transport of neurotransmitters in a Na⁺ and chloride ion (Cl⁻) dependent mechanism (Chen, et al., 2004). In addition to their functional similarity, SLC6 proteins are anticipated to possess a similar structure as they share a

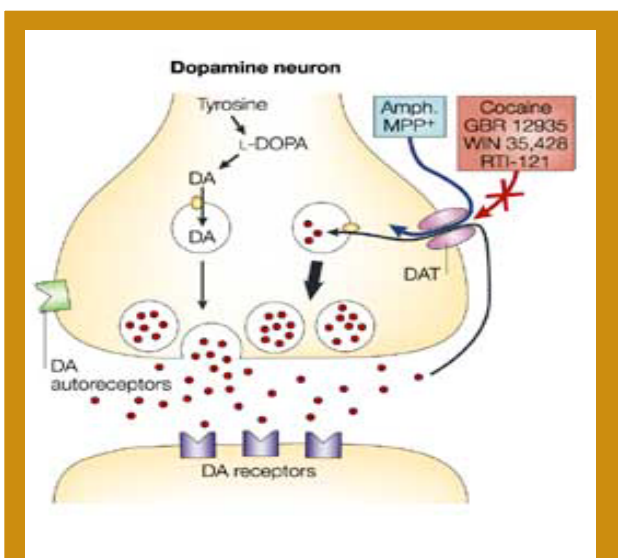


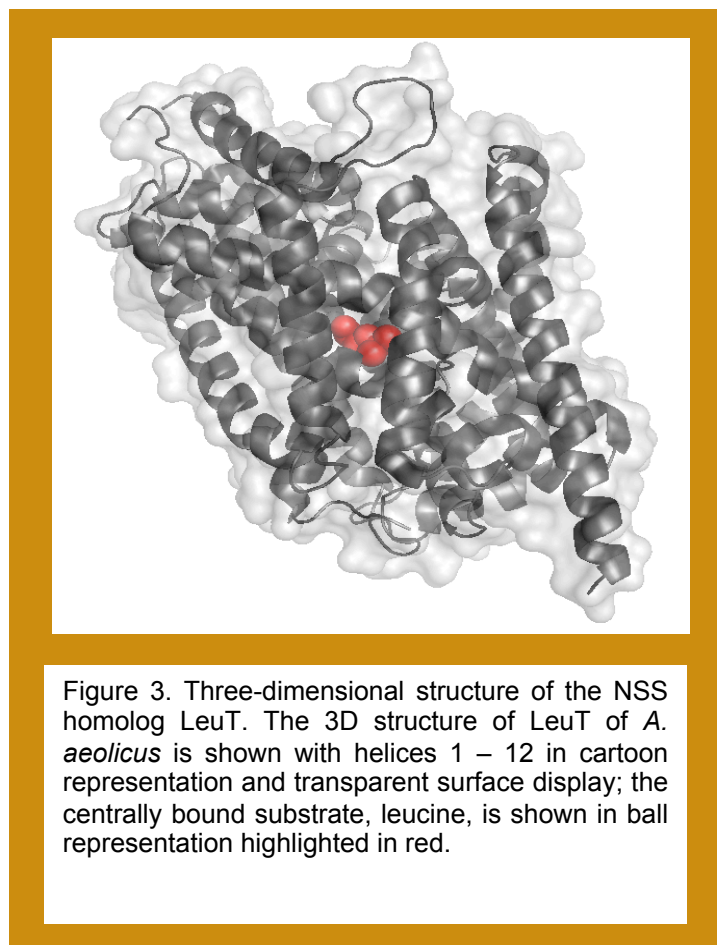
Figure 2. The dopamine synapse. Dopamine (DA) is accumulated in vesicles in the presynaptic neuron and released in the synaptic cleft where it can bind to DA receptors located in the postsynaptic neuron. Clearance of DA from the synapse is achieved by re-uptake of DA into the presynaptic neuron by the dopamine transporter (DAT). Psychoactive drugs, e.g. cocaine or amphetamines, inhibit the re-uptake leading to increased levels of DA in the synapse.

high degree of similarity in their amino acid sequence. It was expected that these proteins consist of hydrophobic α -helices that span the membrane in zigzag fashion and are connected with hydrophilic loops. However, the properties of a protein also differ based on the composition of its amino acid residues, in particular, it is anticipated that the neurotransmitter translocation pathway within the different NSS members is formed by the similar positioning of α -helices in the proteins. On the other hand, subtle differences in the residue arrangement are responsible for the specific transport of a particular neurotransmitter (Anderson Wang & Lewis, 2010).

Biogenetic studies on SLC6 transporters using LeuT

An innovative breakthrough in the understanding of the structure-function relationship of NSS members came from the Gouaux laboratory by determining the high resolution crystal structure of the *Aquifex aeolicus* NSS homolog LeuT (Yamashita et al., 2005), the first NSS member for which crystallographic structure data are available. LeuT, a leucine transporter (Fig. 3), has become the primary template for studying SLC6 transporters due to its sequence similarity with its human counter-

parts. The advantage of LeuT as useful NSS model system over mammalian NSS members is due to the fact that bacterial homologs are more amenable to genetic manipulation in order to facilitate genetic engineering to produce large amounts of pure protein required for crystallization. Many detailed biochemical studies have been carried out to elucidate the molecular mechanism of NSS proteins using LeuT as a model system. LeuT co-crystallized with TCAs (tricyclic antidepressants) provide a glimpse of how drugs act on NSS at the molecular level (Singh et al., 2007). Recently, the serendipitous crystallization of the SLC6 *Drosophila melanogaster* dopamine transporter (dDAT) revealed a similar structural configuration as LeuT (Penmatsa et al., 2013). However, several modifications were required to obtain diffracting crystals of dDAT, leading to a transport-inactive protein variant that complicates the interpretation of the effect of the TCA (nortriptyline) identified in the primary substrate site in dDAT. Using the high-resolution LeuT crystal structure as template for computational simulations in conjunction with biochemical and radiotracer flux and binding studies, comprehensive studies can be performed that to date provided unprecedented information

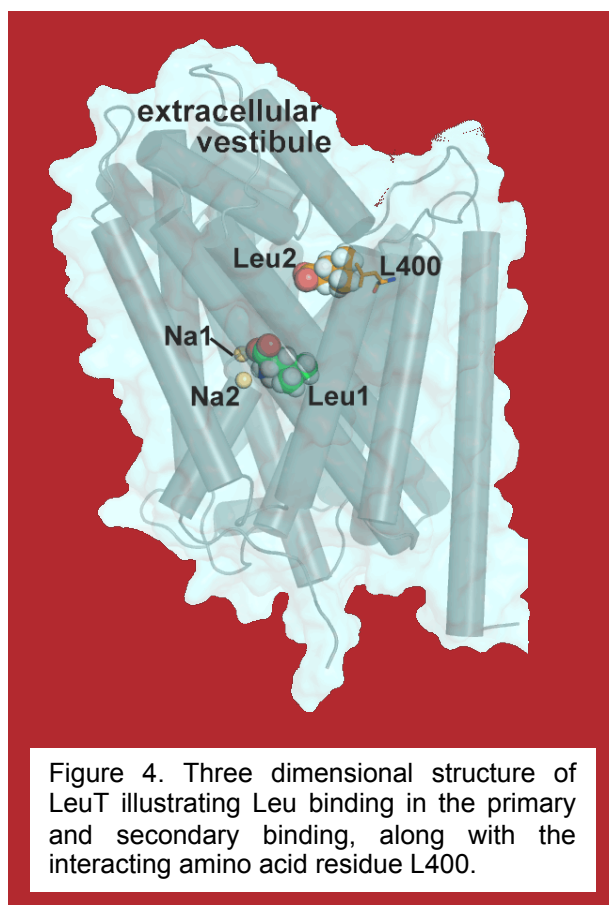


about the mode of action of these medically important proteins (Shi et al., 2008).

Alternative modeling of the LeuT transport mechanism with a second binding site

Studies carried out by the Quick lab proposed an innovative transport mechanism involving the binding of the 2 Na⁺ (in the so-called Na1/Na2 sites) required for the transport of leucine (i.e., 2 Na⁺: 1 leucine molecule) across a membrane (Shi, et. al, 2008). Previous studies done in the Quick lab showed that the binding of Na⁺ is required for the conformational changes that occur within the pro-

tein allowing it to shift from the “outward-facing” conformation position (where binding sites are exposed to the synapse), to the “inward-facing” conformation (where binding sites are exposed to the cytoplasm and the substrates are released) creating the transport (Quick et. al, 2006). More recent studies using steered molecular dynamics (SMD) simulations together with ample of substrate binding and transport studies (Shi et. al, 2008) suggest that in order for Leu to be transported, the substrate must bind to LeuT in the primary (S1) binding site (observed in crystallization, see Fig. 3) and second substrate (S2) binding



site located about 11 Angstrom (Å) over the S1 site (Fig. 4). The S2 site overlaps with an extracellular vestibule that was shown to bind human drugs (see paragraph below). The S2 site is comprised of 2 parts: a hydrophobic pocket composed of Leu29, Tyr107, Ile111, Trp114, Ala319, Phe320, Phe324, and Leu400 which accommodates the Leu hydrophobic side chain, and a polar region composed of the Asp404 and Arg330 (Fig. 4). Allosteric interaction between the network of the two Na⁺ and two Leu sites, particularly the binding of Leu in the S2 site, is responsible for triggering the release of Na1 and Na2 into the cytoplasm (Shi et. al, 2008;

Quick et al., 2009).

LeuT Binding TCAs

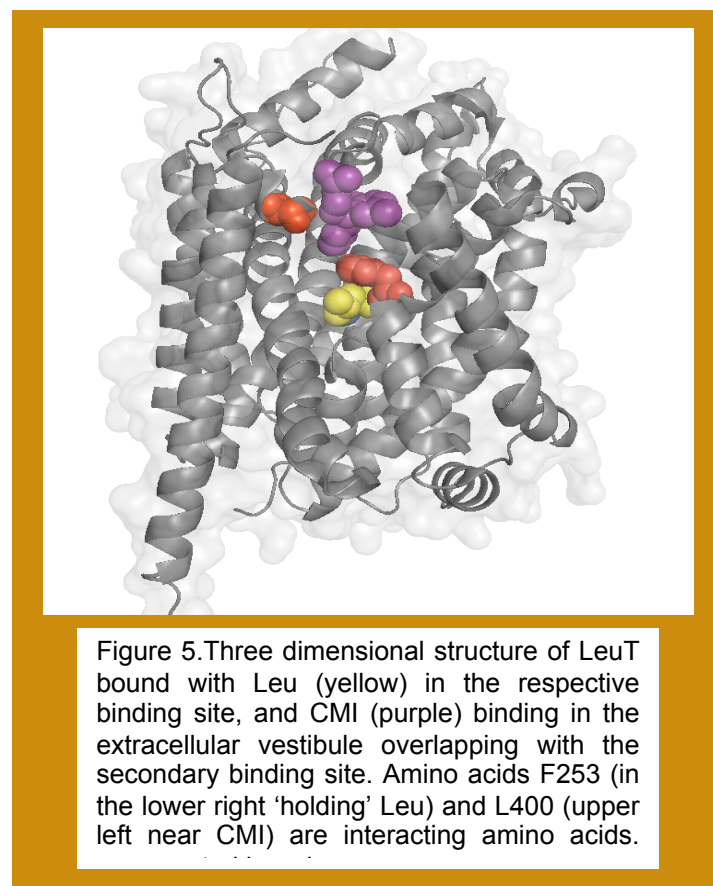
Until very recently, studies done with drugs used in the treatment of psychiatric disorders concluded that these psychoactive compounds compete with substrate for binding in the S1 site. However, recent crystallographic evidence reveals that some of these drugs, such as TCA's (tricyclic antidepressants) bind to an extracellular vestibule (Singh et al., 2007; Zhou et al., 2007). Since this extracellular vestibule and the S2 site in NSS overlap (see Fig. 4), it has been postulated that the inhibitory action of TCA's is based on the disruption of the transport mechanism by blocking

access of substrate to the S2 site (Shi et. al, 2008). When Leu and 2 Na⁺ are binding with LeuT simultaneously with a TCA, the position looks almost identical to the original crystal structure because Leu and Na⁺ cannot bind to secondary site in the extracellular vestibule. The binding of a TCA (such as clomipramine, CMI), while it occupies the second binding site, produces the same effect as mutated or dysfunctional secondary binding site (Fig. 4, Fig. 5) with respect to the transport of Leu, therefore consistent with the behavior of transport inhibitors (Shi et. al., 2008).

Statement of Purpose

Previous studies have shown that the interaction

of CMI with LeuT impairs substrate transport by this NSS member. Due to the fact that CMI was found in a location that physically overlaps with the S2 substrate binding site it was postulated that binding of CMI binding prevents substrate binding to the S2 site, thereby ablating substrate transport. Using direct radiolabeled Leu binding studies it is hypothesized that CMI inhibits transport by directly competing with substrate binding to the S2 site but not with the S1 site and that higher Leu concentrations can (partially) overcome the inhibitory effect of CMI. To dissect these molecular events, binding of Leu was measured in the presence or absence of CMI to Leu-WT and two



different mutants, each with a mutation of an amino acid that interacts with Leu in the S1 or S2 site (LeuT-F253A and L400S, respectively, Fig. 5). (...)

Results and Discussion

Fig. 6 shows that in the absence of CMI LeuT-WT was able to bind more Leu than the mutants, regardless of whether the assay was performed at 3H-Leu concentrations of 100 nM or 1,000 nM. At 100 nM 3H-Leu, both mutants could only achieve nearly 50% binding when compared to WT (note that the raw data were normalized with regard to the highest WT value). This is consistent with the hypothesis that the mutations within the primary and secondary binding sites of LeuT selectively impair binding to a particular site. Similar results were observed at a concentration of 1,000 nM 3H-Leu, only with slightly more variation; when CMI was added to 1,000 nM concentration the percent of binding in WT and F253A appear to be slightly higher by roughly 10% than when CMI was added to the 100 nM concentration.

Adding CMI simultaneously with Leu caused the amount of substrate binding to WT to drop by approximately 50% due to the interaction of CMI in the S2 site. At 100 nM, the mutants showed a greater

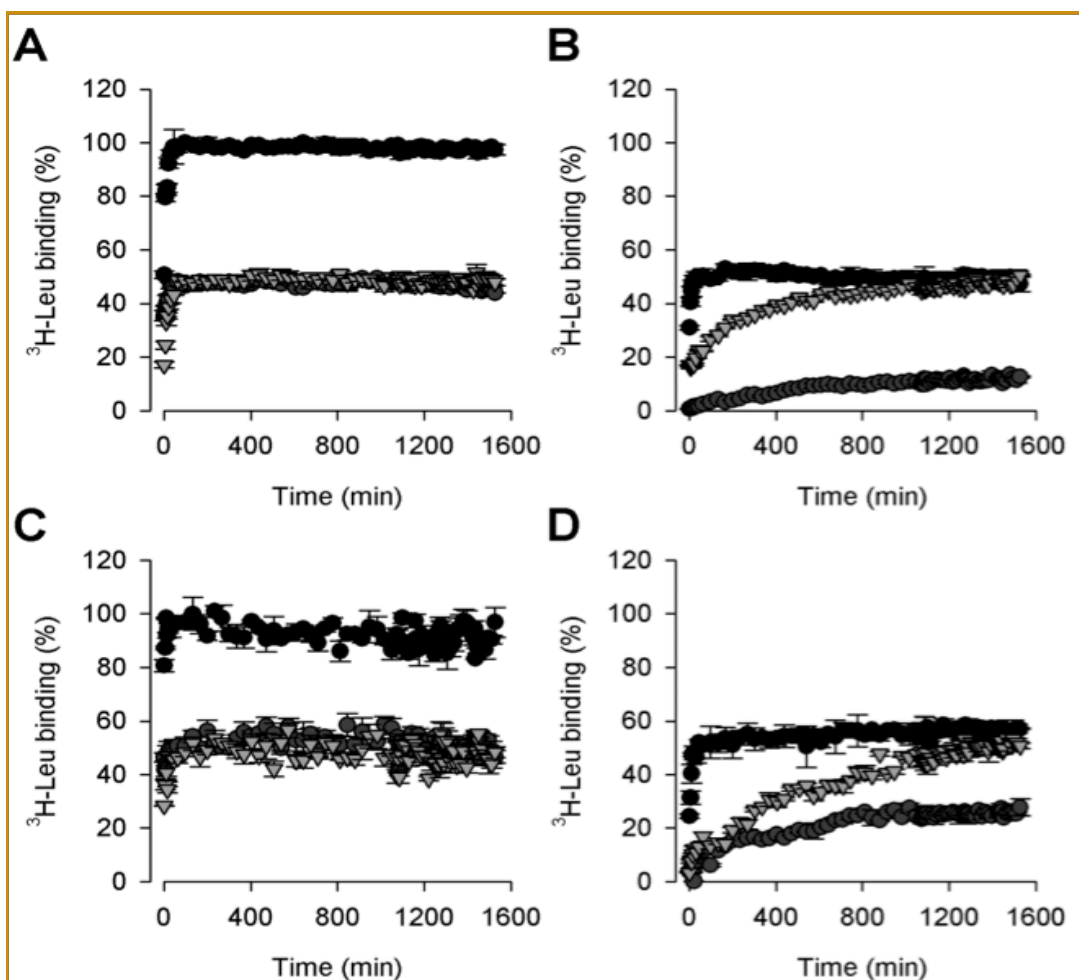


Fig. 6. 3H-Leu binding to LeuT-WT, -F253A, and -L400S. 3H-Leu binding was tested at concentrations of 100 nM (149 Ci/mmol; panel A and B) or 1 μ M (9 Ci/mmol; panel C and D) in the presence (panel B and D) or absence (panel A and C) of 1 mM CMI. 50 ng of purified protein variants (LeuT-WT, $\bullet$; -F253A, $\blacksquare$; R-L400S, $\blacktriangle$) were used per 100- μ L assay. Data points represent the mean $\pm$ s.e.m. of triplicate determinations. CMI was added with 3H-Leu (simultaneous addition).

differentiation in percent of binding between each other and WT than they did in the absence of CMI. The activity of L400S became consistent with WT at 1400 mins, at about 50% of Leu binding, whereas the activity of F253A remained lower, maxing out at only 20% binding at 1600 mins. The differentiations observed in binding that occurred between the mutants can be explained by their positioning when interacting with the substrate. F253A is located in the primary binding site (see Fig. 4),

the positioning of F253A when Leu is binding suggests a heavy involvement required for the substrate to bind (in Fig. 5, it almost appears to be embracing the Leu). L400S on the other hand (see Fig. 5), although does appear to be interacting with binding occurring in the extracellular vestibule, there is not as heavy competition in F253A. Because F253A has a greater involvement in the binding of Leu than L400S, when it is mutated, less binding can occur. From these data collected, it is plausible to assume

CMI competes with Leu binding in the S2 site. The data shows that Leu binds 50% less effectively to WT in the presence of CMI because when S2 is occupied with the inhibitor, Leu can only bind to the protein in the S1 site. Experiments with mutants F253A and L400S in the absence of CMI where the S1 or S2 binding sites were disabled showed 50% less binding than WT. When Leu and CMI were simultaneously added to WT, a 50% decrease in binding was observed making it a valid assumption to

suggest CMI impairs the binding of Leu the same way disabling the S2 does in non-mutated LeuT protein.

Based on the results from adding CMI simultaneously with Leu, it may also be concluded that although CMI does impair the binding of Leu through direct competition at S2, when tested at concentrations of 100 nM and 1,000 nM Leu can still partially bind to LeuT, although there is a significant 50% decrease in binding. To test whether higher concentration of Leu can ‘overcome’ the inhibitory effect of CMI in the S2 site we performed a similar experiment in

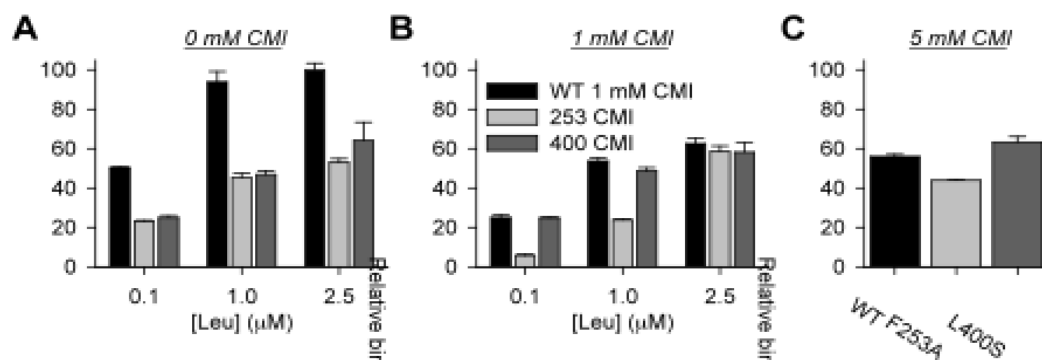


Figure 7. Leu binding to LeuT-WT, -F253A, and -L400S in the absence of CMI (panel A), 1 mM CMI (panel B), or 5 mM CMI (panel C). 3H-Leu binding was tested at concentrations of 0.1 μ M (149 Ci/mmol), 1 μ M (9 Ci/mmol), and 2.5 μ M (9 Ci/mmol) to purified protein variants. Each graph shows data normalized with respect to WT binding at 2.5 μ M 3H-Leu in the absence of CMI (panel A).

which the effect of CMI on binding of 0.1, 1, and 2.5 μ M Leu was assessed.

Figure 7. Leu binding to LeuT-WT, -F253A, and -L400S in the absence of CMI (panel A), 1 mM CMI (panel B), or 5 mM CMI (panel C). 3H-Leu binding was tested at concentrations of 0.1 μ M (149 Ci/mmol), 1 μ M (9 Ci/mmol), and 2.5 μ M (9 Ci/mmol) to purified protein variants. Each graph shows data normalized with respect to WT binding at 2.5 μ M 3H-Leu in the absence of CMI (panel A).

In the second experiment, the results of 3H-Leu binding at various concentrations to the proteins in the absence of CMI

(Fig. 7, panel A) were consistent with findings of the previous experiment (Fig. 6 A and C); mutants exhibit roughly 50% of the level of binding observed for WT at their respective 3H-Leu concentration because one of two (either S1 or S2) sites are impaired by a mutation, thus resulting in 50% of binding.

Panel B is also consistent with the results from the previous experiment—when 1 μ M CMI is added to WT, there is a 50% decrease in binding in WT for each 3H-Leu concentration when compared to its respective percent binding in absence of CMI (black bars only, Fig. 7 panel A compared with panel B). For the L400S mutant, the addition of 1 mM CMI has no effect. This observation supports the hypothesis that Leu binds only in the S1 site because L400S already has an impaired S2 site caused by the mutation; binding of CMI to

the extracellular vestibule that overlaps with the S2 site exhibits no inhibitory effect. The 50% reduced binding observed occurs in the L400S’s intact S1 site. This also causes L400S-mediated binding to resemble WT binding in the presence of CMI where only one out of two sites is available to properly bind Leu. Panel B also shows major decreases in binding of F253A in the presence of CMI at 0.1 μ M – this mutant has a dysfunctional S1 site, and in the presence of CMI, both the S1 and S2 sites are impaired to bind Leu. However, when the concentrations of 3H-Leu are increased to 1 μ M and 2.5 μ M, Leu can achieve higher percentages of binding to F253A. It is possible that when CMI is bound to the S2 site in F253A, the higher concentration of 3H-Leu “floods” the mutated S1 site, which would result in higher level of binding, although additional testing is required

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to investigate this claim.

Panel C further indicates that CMI binds in the S2 site and therefore does not prevent Leu from binding in the S1 site because when the concentration of CMI is raised to 5 mM, percentages in binding of Leu to the proteins remains the same when compared to 1 mM CMI (panel B). As indicated by L400S, the percentages remain static at roughly 50% of total binding, therefore suggesting higher concentrations of CMI do not prevent Leu binding to the S1 site and is specific to Leu binding to the S2 site.

Conclusion

By understanding the specifics of the functional binding patterns of Leu, more can be revealed about the mechanism that would allow for the manipulation of protein interaction. Investigation and further

discovery about the binding mechanism of NSS provides a glimpse of how psychoactive drugs such as TCA's and other drugs are influencing the human body at the molecular level. With a better understanding of the mechanism by which psychoactive drugs act on the NSS transport mechanism further advancements towards improving medical treatment for patients with disorders such as depression or addiction can be developed. Understanding this mechanism can also lead to treatments for diseases that mirror this kind of molecular transport modal, which could potentially be targeted by other drugs and used for future treatments.

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Nanoparticles: Synthesis of a Low Cost Complex Multifunctional and Surface-Switchable Nanoparticle Platform for Metastasized Cancer Treatment, by Jazz Munitz (Junior)

Abstract

In recent years, the field of Nano medicine has attracted much interest by the bio-medical community. (Jarzyna et al., 2009) Nano medicine, or the “development and application of nanoparticles for biomedical purposes”, reaches a broad range of fields of study, using particles ranging from 2-100

nm in size. Fields of research such as iron oxides, quantum dots, as well as gold nanoparticles are being recognized and utilized for their abilities in “diagnostics as well as therapeutics”. In recent years, Nano medicine has been the center of the scientific community resulting in many new advances in the fields of facilitated mo-

lecular imaging, (Jarzyna et al., 2009) targeted drug delivery, as well as treatments for many diseases facing our society of humanity in today's time. The combination of these nanoparticles with tumor-targeting affinities, smart release systems, and drugs that have cancer-killing abilities are a viable scheme for cancer treat-

ment.

Introduction

Many discoveries in other bio-medical fields have made the success of this research possible. One discovery, the enhanced permeability and retention effect, or EPR, is a strange and useful phenomenon when developing and constructing nanoparticles for the use of tumor tar-



getting. The EPR effect, discovered by Matsumura and Maeda in 1986, describes a process in which “large gaps exist between endothelial cells in tumor blood vessels”. In order for tumors to grow at rates that surpass rates of growth demonstrated by normal body cells, they must gain nutrients in larger volumes. The tumor cells stimulate the growth of new blood vessels through the vascular endothelial growth factor, or VEGF, as protein tumor cells, as well as normal cells produce in order to stimulate the process of both vasculogenesis as well as angiogenesis. Both processes stimulate the growth of new blood vessels. In normal body cells, and body function VEGF is used to grow new blood vessels when old ones have been broken down or damaged through regular exercise or injury. How-

ever, tumor cells utilize this process to gain more nutrients than the normal cell, in order to stimulate rapid growth, while other cells receive fewer nutrients, contributing to the disease. Through the overuse of VEGF, vascular diseases and disorders can develop, further adding to the damage tumor cells can cause on the

body. Although drugs, like bevacizumab can contribute to the stopping of tumors growing blood vessels, (Fang, Nakamura, & Maeda, 2010) the EPR effect proves very useful in finding and treating tumors through the use of nanoparticles.

Matsumura and Maeda observed and recorded, “Most solid tumors therefore exhibit enhanced vascular permeability, which will ensure a sufficient supply of nutrients and oxygen to tumor tissues for rapid growth. The EPR effect considers this unique anatomical-pathophysiological nature of tumor blood vessels that facilitates transport of macromolecules into tumor tissues...this EPR effect-driven drug delivery does not occur in normal tissues.” (Fang, Nakamura, & Maeda, 2010) In developing a drug that

can target, and in future advancements in the field, treat the tumor tissue, it is crucial that cells targeted are only tumor cells, as in both fields of study, healthy cells are not useful to image, or kill. The EPR effect gives nanoparticle developers and researchers an understanding of how to develop a particle that will reach the tumor cells, and not normal healthy cells.

A successful nanoparticle was developed by the Nanomedicine team at the Mount Sinai Trans Molecular Imaging Institute, the Clinical Institute in Denmark, the Angiogenesis Laboratory at VU University Medical Center, the Department of Gene and Cell Medicine and Mount Sinai School of Medicine, and the Monzino Cardiology Center in Milan, a Cy5.5-PEG-DSPE lipid based particle was developed for an enhanced imaging catalyst. Through the utilization of the EPR effect, previous research in Nano emulsions, and understanding of size and its relation to various tumors, a usable particle was developed. (Jarzyna et al., 2009)

In the beginning research of Nanomedicine and its uses in tumor imaging and treatment, one great boundary had to do with the originally thought to be lack of control over particles once they were administered into the

body. With surgery, and other externally controlled treatments for removing tumors, doctors can see and control the processes and functions happening in the body during the treatment. However, with treatments on the Nano scale, the processes taking place once the drug has been administered cannot be controlled by doctors. Therefore, during development of said drug, the particle must be designed in a specific and precise way, assuring that the particle can find the tumor and perform its function without human assistance and control. In the development and synthesis of the previously discussed particle, researchers and developers instituted various timed out processes in order for the particle to function properly. (Cormode, Jarzyna, Mulder, & Fayad, 2010)

Developed by researchers at the Translational and Molecular Imaging Institute, SyMo-Chem in the Netherlands, Cardiovascular Research Center and Mount Sinai School of Medicine, The Laboratory of Nano medicine and Biomaterials, Department of Anesthesiology, Brigham & Women’s Hospital, Harvard Medical School, Department of Chemical Engineering at the Massachusetts Institute of Technology, in Cambridge, the Department of Vascular Medicine, in Amsterdam,

the complex multifunctional and surface-switchable nanoemulsion platform is a first of its kind. The challenges previously discussed with nanoparticle development and utilization has been overcome due to many inventions and new implementations demonstrated in this particle. “To deal with the aforementioned limitations of ligand functionalized nanoparticles we have developed a methoxy-polyethylene glycol (PEG)-lipid that can be cleaved by matrix metalloproteinase-2 (MMP2).” [An enzyme released by tumor cells during the metastasis process] “Incorporation of this cleavable PEG-lipid into a multifunctional nanoemulsion platform renders nanoparticles with a surface-switchable coating...shielded by the cleavable PEG-lipids...chosen MMP2 cleavable sites as the enzyme is highly expressed at...solids tumors and atherosclerotic plaques.” PEG-lipid coating will then be removed after an accumulation or particles around site of tumor causes the coatings to be removed when exposed to MMP2. Thus, the next step in the release of the substance suspended within the soybean oil will follow suit. (Gianella et al., 2011). Another obstacle in the synthesis of such a nanoparticle lies in the production of such. Due to the

size of said particle, many are needed for a single administration of such a drug into a patient. In order to mass-manufacture the particle, developers of the surface-switching nanoemulsion developed a self-assembling construction, allowing for efficient production of particle. To do this, they utilized the amphiphilic properties of phospholipids, which form the barrier of the particle. A phospholipid is a molecule that has amphiphilic properties, meaning that it is both hydrophobic and hydrophilic. The structure of the phospholipid contains a hydrophobic tail, and a hydrophilic head. The head is hydrophilic, since it is polar, which makes it attracted to the polar nature of water. The hydrophobic tail points itself toward the inside of the particle, because it contains oil, which is nonpolar. The reason the tail is hydrophobic and nonpolar is because of the carbon chain that forms the tail, and the occasional double bond resulting in the “kick” 3d shape. The hydrophilic head points itself outwards towards the water environment. The polarity is caused by the phosphate groups that give the head a charge. The polarities of the phospholipids lock themselves together forming a fatty barrier containing the soybean oil, and proteins suspended within. The size

of the over-all particle can be altered by adding or releasing the volume of oil inside, and correspondingly adding or getting rid of the number of phospholipids present that forms the barrier. (Jarzyna et al., 2009)

A self-assembling, surface switchable and multifunctional nanoemulsion particle opens up many doors for the future of this research and opportunity in the field of utilizing nanoparticles for the use of tumor imaging, as well as therapy. This particle, the most recent in Nano medicine endeavors of its kind, can be replaced with different drugs or substances suspended in the soybean oil, to be delivered to the tumor tissues. Due to the flexibility of the particle, both structurally and from an opportunity stand-point, further advancements in therapy and imaging can be developed. Due to the dual functioning nature of this particle, as it can be utilized for both imaging and treatment, from the time of administration, through the treatment, and post-therapy, the particles movement and work can be observed through CT and

MRI imaging. (Jarzyna et al., 2009)

Where this particle stands out from others in its class, is that it includes a “a lipophilic therapeutic...renders this platform therapeutic.” This means that in the case of the utilization of stem cells or other patient-specific, tailored treatment, such a personalized drug or therapy could be instituted in synthesis, and administered through the particle. Another breakthrough with this new platform has to do with its possible future and ability to treat diseases other than cancer. If the enzyme and cleavable agent were to be switched

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to a pair suited for other diseases, the nanoemulsion could be altered in synthesis, or in size, (as it can currently be stable in sizes 30, 60, and 95 nm), in order to fulfill the specific hydro-environments of other diseases in other regions of the body. (Jarzyna et al., 2009)

The incorporation of various natural compounds can facilitate the arrest of cancer cells, and can eradicate the disease from the body. In this study, the natural compound used (name of compound retracted due to proprietary information) exhibits spe-

cific biochemical and biological abilities and structure which make it suitable for a cancer drug. This compound is extremely hydrophobic, a necessary characteristic that allows it to be integrated in the hydrophobic PLGA core of monolayer micelle nanoparticles. This compound has a natural ability to induce cell apoptosis through the facilitation of the “caspase cascade” by which cells essentially commit suicide by destroying structure, genetic information, and all other components. This process can be instigated by a sin-

gle caspase introduction, meaning that very little volume of the compound is necessary in each nanoparticle to induce cancer cell death. This in turn will drive cost of therapy production down, increasing the availability of this therapy to all patients regardless of financial hindrances.

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The Promise of Gasification as an Alternative Energy Resource and the Potential for the Use of Gasification Byproducts.

by Aidan Datené (Junior)

Introduction

In the United States, about 225 million tons of municipal solid waste (commonly referred to as garbage) is generated annually. One third of this

waste is either recycled or composted. Landfilling or incineration manages about 150 million tons of municipal wastes. Landfilling faces increasing opposition because of its drastic effect on the environment (Sallam, 2012). Increasing demands for fuels and electricity have coupled with this fear, and a heightened awareness of the influence of various pollutant species (including greenhouse gases) on the global climate system has created the incentive to develop alternative energy sources (Sallam, 2012). The consumption and depletion of Earth's natural resources (minerals,

fossil fuels, and ecosystems) has contributed to global warming and loss of biodiversity. The 21st century coming of age science of industrial ecology is defined as the “re-thinking and re-designing [of] industrial activities with full knowledge of their environmental impacts (Themelis, 2007),” and is primed to find sustainable ways of handling the billions of tons of waste solids created by humans (Themelis, 2007).

Gasification is becoming a prominent alternative to landfilling and basic methods of incineration (Butterman, Castaldi, 2008). Gasification breaks down the composi-

tion of biomass (biomass is carbon based with organic molecules), plastics, mixed waste materials, and municipal solid waste, and creates a desirable “syngas gas” or synthetic gas along with byproducts including ash and char (Leonard, 2012). Gasification is a thermochemical process that consists of two main stages, not including the refinement of products (Klein, Themelis, 2003). Pyrolysis, the first stage, occurs at temperatures below 600°C (1112 °F). Pyrolysis is the initial gasifying of the feedstock, allowing volatile components (parts of organic compounds) to be released (Klein, Themelis,



2003). This devolatilization process produces volatile gases, char, and ash materials (Pantelides, 2006). In the second stage, the carbon in the ash and char are introduced to and treated with a co-reactant, oxygen, steam (water vapor), or carbon dioxide, resulting in the producer gas “syngas” (Klein, Themelis, 2003). The exothermic reaction in the gasification char and ash, between the carbon and the co-reactant, provides thermal energy that drives the pyrolysis phase. The reactions that create syngas are either endothermic or exothermic, and their rates depend on pressure, temperature, the co-reactant, and co-reactant concentration. The product gas can be used as fuels in steam boilers, reciprocating engines, and combined cycle turbines (Klein, Themelis, 2003).

Gasification products can be controlled; removing metal ions, tars, and oils during the thermochemical process and during the product’s refinement, results in a reduction of carbon dioxide emissions (Themelis, 2007). Recovering energy from municipal solid waste in Waste to Energy Plants (such as gasification facilities) can reduce landfilling as well as air and water emissions, further lessening dependence on fossil fuels for power generation (Klein, Themelis, 2003). The pro-

duced syngas is primarily composed of hydrogen and carbon monoxide, desirable molecules in creating fuel. During oxidation of biomass the Boudouard reaction ($C+CO_2$ to $2CO$), the Water Gas Shift reaction ($CO+H_2O$ to $H_2 + CO_2$), and the reverse Water Gas reaction ($C+H_2O$ to H_2+CO) take place, resulting in the gain of creating preferable molecules for energy production when refined (GREN-WHICH). Refinement includes fuels, notably gasoline, and electricity among others (Butterman, Castaldi, 2008).

The gasification process is a self-sustainable process. Studies have concluded that gasification has beneficial capital gains and is an ecologically ample process. According to a recent study, the gasification process has been seen to cost less per kWh of electricity generated than that of combustion plants (Klein, Themelis, 2003). This attests to gasification’s higher efficiency of converting thermal to electrical energy once feedstock components are converted into synthetic gas (Klein, Themelis, 2003). These are encouraging numbers that can cause a prevalent use of the process in place of other, biomass to energy plants.

Gasification of biomass is a growing because of its positive attributes. Biomass maintains a carbon

neutral status, meaning it maintains a zero carbon footprint (Butterman, Castaldi, 2009). This makes biomass a prime candidate for gasification because there would be no carbon emissions in any form, when biomass is oxidized during pyrolysis (beginning phase of gasification). Predictions made from current studies suggest that if 20% of liquid fuels were produced from a carbon neutral source, such as biomass, there would be a resounding 15% reduction in CO₂ emissions (Butterman, Castaldi, 2009). Total global CO₂ emissions amount to nearly 29.5 billion metric tons annually. One third of these emissions come from burning gases in the transportation sector equivalent to 1.4 billion metric tons, which so can be averted through fuel replacement alone (Butterman, Castaldi, 2009). A resounding statistic, “for a typical automobile producing 6 tons of CO₂ /per year, this would be equivalent to removing 308 million vehicles from the road annually (Butterman, Castaldi, 2009)” provides great optimism for the future of gasification

with biomass as the world looks to control its impact on the environment.

Biomass fuels are globally available (Butterman, Castaldi 2007). Biomass does not have the intermittency associated with wind and photovoltaics (wind and solar converting methods), and they constitute nearly one quarter of the fuel available from municipal waste. Biomass fuels currently provide 3% of the U.S. energy production. Nearly one third of the renewable energy sources in the United States can be attributed to woods, grasses, and agricultural forestry wastes, and residues (Butterman,

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Castaldi 2007). On the basis of a recent study, biomass has been found to be capable for supplying 5% of the nation's power by 2030. Biomass derived fuels have the potential to provide 20% of the U.S. transportation (Butterman, Castaldi 2007).

Currently there are limited uses for the byproducts of gasification, ash or char (Morf, 2012). These byproducts of the gasification process are generally discarded in a landfill (CEWEP). Recently char and ash have been used in construction applications or as an alternate cover for landfills (CEWEP). However, the majority is still discarded. Studies by Morf (2012), Gori (2009), Sallam (2008), and Klinghoffer, Castaldi, and Nizhou (2012), focus on sundry uses for these byproducts and their beneficial properties.

Char is a residue that is produced during the pyrolysis phase in gasification (Klinghoffer, Castaldi, Nizhou, 2012). Char is composed of material from the original feedstock that was not successfully converted to syngas for fuel production. Char has been tested for catalytic properties and performance in an effort to replace commercial catalysts by efficiency serving as a cheaper alternative (Klinghoffer, Castaldi, Nizhou, 2012). This was tested under the hypothesis that char is de-

pendent on gasification conditions. When char samples of biomass gasification are treated with different co-reactants, they release various amounts of carbon (which has been used in catalytic applications), and can chemically convert tars to useful products by breaking down the sample's composition (Klinghoffer, Castaldi, Nizhou, 2012). Results found were favorable; all parts of the hypothesis were confirmed, and optimistic results have char replacing commercial catalysts under certain conditions (Klinghoffer, Castaldi, Nizhou, 2012). Analyzing chars that were created under different temperatures with different co-reactants, (reactants such as water and carbon dioxide were selectively added to the pyrolysis phase), demonstrated that the surface area of char could be modified by changing gasification conditions. Results continued to show that char was able to break down hydrocarbons methane, propane, and to an extent, nitrogen (Klinghoffer, Castaldi, Nizhou, 2012). The char proved high efficiency when breaking down aliphatic bonds present in methane and propane. This performance was measured against two commercial catalysts and the char had superior results between 500 and 900 degrees Celsius (sample

H₂O-750-60). When not under these temperature constraints, both H₂O and CO₂ samples under 500 degrees did not provide adequate results, and above 900 degrees Celsius H₂O samples experienced sintering; the breaking down of the sample's microporous network while those that were produced under CO₂ did not provide satisfactory results (Klinghoffer, Castaldi, Nizhou, 2012).

Recent advances in recovering precious metals and rare earth elements from bottom ash have been cultivated from municipal solid waste (Morf, 2012). Efforts to recover these metals and elements have recently increased. This is not a relatively new concept. Law and Gordon (1979) found that there were considerable amounts of silver concentrations in bottom ash (38 ± 8 mg/kg). In 1996, Simon wrote a cover article for a Swiss paper stressing the economic potential for valuable metals that are present in bottom ash, "which showed 40-400 times higher levels of Ag [silver] compared to earth crust levels (Morf, 2012)" Although promising at the time, concentration levels were considered too low to make a recovery of the elements and the metals that made the product economically feasible (Morf, 2012).

In recent years, research

has continued to find economically sound methods of recovering metals and rare earth metal elements because of the correlation with an increase demand for these metals (Morf, 2012). Among investigated techniques, there is a magnetic separation method and an eddy current separation method used to generate iron metal fractions and non-ferrous fractions (metals that are light weight, have a higher conductivity than ferrous metals, and do not corrode) (Morf, 2012). The conclusions of the study proved that it is feasible to recover metals but in small concentrations (direct recovery). However, there were unexpectedly large annual amounts of heterogeneous solid waste in the substance flow, which hindered the ability to extract precious metals and rare earth elements (Morf, 2012). This is an area that needs further research into finding ways to facilitate effective methods of extracting precious subsidies while maintaining an economic equilibrium. Potentially high market values for these metals and elements provide optimism. A large reduction on environmental impact by means of future waste and resource management systems may be achieved for economic revenue orchestrated by the high trading value of such metals as gold and silver, provided

that efforts to develop recovery techniques are sustained (Morf, 2012).

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The Effect of Mindfulness-Meditation Practice on Anxiety Levels Among High School Students, By Jordan Hudock. (Junior)

Hypothesis:

Participation of high school student in mindfulness meditation practice will reduce the average level of anxiety in students.

Purpose:

With the nonstop, always on the go, modern lifestyle almost all of us face, everyday anxiety has become

an epidemic. In America, reportedly seventy-seven percent of all people are not able to cope with this stress or manage it in their daily lives (Millar, 2010). An even more shocking ninety-seven percent of college students report that they experience anxiety on a daily basis as described in the same study (Millar, 2010). If something

beneficial, not only to the students' stress levels, but also to their concentration and awareness levels, could be implemented in school systems, shouldn't it be? Study after study shows meditation to significantly alleviate anxiety symptoms (Orme-Johnson et al., 2013) along with a reduction in sleepiness throughout the day and

an increase in focus and awareness (Lee et al., 2007). If this simple practice could be implemented in schools' physical education classes, imagine the widespread benefits that would take hold. This study will be done to collect concrete data on meditation's benefits when in the school setting.



Background:

Anxiety levels can be classified using psychological, physiological, or biological responses or lack thereof. When a person is experiencing anxiety, psychological responses include feelings of worry, nervousness, or unease, typically about an impending event or something with an uncertain outcome (Roth, 1994). Key examples of physiological response to anxiety include a lack of focus, an inability to unconsciously remain still, rapid breathing, trembling hands, cold hands and feet, a rapid heartbeat, a dry mouth and overall restlessness (So et al., 2001).

During periods of anxiety, the muscle demands higher levels of glucose and oxygen running through the bloodstream. For this to be possible the heart has to pump faster and blood pressure must increase.

on the surface of adrenal cells, which initiates a complex process that turns cholesterol into cortisol. When produced, this hormone is pumped into the body and attaches to its receptors. At this point, cortisol can interact with other cells to increase the sensitivity of the heart and blood vessels. This leads to the effects of epinephrine and norepinephrine. These hormones stimulate receptors in heart cells, which increase the rate of the heartbeat and the force with which the heart muscles contract (Shapiro et al., 2008). Cortisol also ensures that the blood vessels are receptive to epinephrine and norepinephrine. These hormones attach to specific proteins, which cause the narrowing of blood vessel walls. This brings about an increase in blood pressure and allows blood to be sent to essen-

The entire process starts when sensory cells alert the hypothalamus of a potential danger. Cells in the hypothalamus transmit a signal to the pituitary gland, which releases the adrenocorticotrophic hormone (ACTH) to the adrenal glands (So et al., 2001). ACTH signals to both adrenal glands to produce more cortisol. ACTH molecules bind to receptors

tial organs and muscles. Meditation is commonly associated with reducing the symptoms of anxiety. It is a form of contemplation, reflection, or heightened awareness. Alterations in brain function during meditation have been recorded using single photon emission computed tomography, electrophysiology, PET and functional magnetic resonance imaging (Travis, 2001). Although results differ slightly between forms of meditation, overall results indicate intensified signals in brain regions related to attention control and affect regulation, with an increase in dopamine release.

One study, entitled Effects of Transcendental Meditation practice on brain functioning and anxiety reactivity in college students, was executed to determine the effects transcendental meditation practice had on brain function and anxiety levels in college students (Oman et al., 2008). This trial investigated the effects of Transcendental Meditation (TM) on broadband frontal coherence, preparatory brain responses, and power ratios, all of which were measured using the Brain Integration Scale. Fifty college students participated in a pretest, then were assigned to learn either TM immediately or after a ten-week post-test. Analyses revealed

significant increases in Scale scores for students who began learning TM immediately, but decreases in students whose learning was delayed. There were significant reductions in sleepiness among immediate-start students with no change in delayed-start students. The trial showed no changes in habituation rates in immediate-start students, but significant increases in delayed-start students. These data support the value of TM practice for college students and, therefore, it would be interesting to research whether or not these effects translate into benefits for high school students, the subjects of this study.

Another study, done in 2007, was focused on determining the effects that meditation practice had on brain function and anxiety levels in participants diagnosed with anxiety compared to healthy participants (Lee et al., 2007). During this trial, forty-eight participants with anxiety disorder were assigned randomly to either an eight-week meditation program or an eight-week education course. Those in the meditation group practiced meditation over that period of time while the education was instructed on how one should respond to one's anxiety. Results were measured every other week using the Hamilton Anxiety Rating Scale (HAM-A), the

Hamilton Depression Rating Scale (HAM-D), the State-Trait Anxiety Inventory (STAI), the Beck Depression Inventory, and the Symptom Checklist-90—Revised (SCL-90-R). This study's results show a larger decrease in anxiety within the meditation group than the education group, the therefore supporting this paper's hypothesis that meditation is an effective alleviator of anxiety symptoms. The results also show an increase in attentiveness among meditation-group participants.

A third study that specifically demonstrates this paper's hypothesis is called: Randomized trial of a meditation-based stress reduction program and cognitive behavior therapy in generalized social anxiety disorder (Koszicki et al., 2007). Its trials sought to see how well meditation worked in anxiety compared with established psychotherapy interventions. Fifty-three patients diagnosed with generalized social anxiety disorder participated in either an eight-week meditation program or twelve sessions of group therapy targeted at the disorder. The meditation group showed significant improvement in their self-measured anxiety at the end of the trial period, supporting the hypothesis that meditation could be used to alleviate symp-

toms of anxiety among high school students.

Various studies were done to support the theory that meditation significantly reduces anxiety levels. One such example is a recent study by Richard Davidson and colleagues (Davidson et al., 2013). This study was based on the research that opening oneself up to rather than resisting possibly painful experiences can actually steer one's mind away from anxiety. Being open to these types of experiences is a documented result of meditation. For this reason, Davidson sought to discover whether or not meditation would steer peoples' minds from stress in this way. During this study, functional magnetic resonance imaging (fMRI) was used in meditators to dissociate neural activation patterns linked with pain. Compared to non-meditators, meditators experienced significantly less unpleasantness.

Anxiety is extremely prevalent in school systems and meditation is a potential tool that could be used to alleviate symptoms. Schools could take a few minutes each day to integrate meditation into classes in productive ways. As demonstrated in the above four studies, this would likely lower the anxiety levels of students and improve their abilities to concentrate and think clearly throughout the

day.

This paper investigates the role mindfulness-meditation practice plays in alleviating anxiety symptoms among high school students. It is hypothesized that participation of high school students in mindfulness meditation practice will decrease the students' average anxiety level. The above four literature reviews exhibit and support that hypothesis. This study will differ from the directly above-mentioned involving college students (Oman et al., 2008) because, with high schools students, there is an age difference, difference in maturity level, difference in the pressures students face (social and educational), among other variables.

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Role of the Tau Proteins in the onset of Alzheimer's Disease, by Clio Lieberman (Sophomore).

Introduction

Alzheimer's disease affects 30 million people worldwide. It is an advance form of dementia, which includes an early onset (EOAD) and a late onset (LOAD) form. EOAD is thought to be genetic, while LOAD is thought to be caused by many different proteins in the brain. The first hypothesis is the amyloid-beta

hypothesis, caused by the 39-42 amino acid chain of the amyloid precursor protein. Another hypothesis is the tau protein hypothesis, which is caused by hyperphosphorylated 383-441 tau proteins in the AD adult brain. This leads to cognitive damage, causing memory loss, speech impairment, inability to perform basic motor functions, and synaptic loss.

essential in normal aging, but in normal aging, BACE-1 uses α -secretase and γ -secretase to cleave APP. APP is the amyloid precursor protein, a "type one membrane glycoprotein" (Nalivaeva et Turner, 2013). α -secretase and γ -secretase cleave APP at different places on the peptide. When α -secretase cleaves APP, it can be absorbed into the cell. When γ -secretase cleaves APP, it is too large to be absorbed into the cell, and the oligomers aggregate and attack the cell.

APP is used in normal aging as well. In the "developing brain, APP is required for neuronal precursor cells to migrate correctly in the nascent cortical plate" (Nalivaeva et Turner, 2013), regulates "synaptic vesicle exocytosis" (Nalivaeva et Turner, 2013), maintains the use of calcium in neurons, which is used in synaptic transmission. The main problem in Alzheimer's disease is the destruction of neurons when APP is not cleaved properly. An-

other problem is the accumulation of APP when there is an overproduction of the protein or when neprilysin (NEP), the APP degrading peptidase, malfunctions or is down regulated. Amyloid beta proteins are toxic and insoluble, which makes it very hard to remove if neprilysin malfunctions.

Amyloid beta proteins also caused malfunctions in the blood brain barrier (BBB), and disrupts cerebral blood flow (CBF). AB causes leakiness in the BBB, which can let toxic macromolecules into the brain. According to Abbott et al. (2010), "leakage of these large molecular weight serum proteins into [the] brain across a damaged BBB can have serious pathological consequences." When there's too much leakiness in the BBB, it can let macromolecules through that are toxic, such as glutamate, which is toxic when in the brain (Bamburg and Bloom 2009). This can disrupt normal neuronal func-



Amyloid beta hypothesis

The first hypothesis is the amyloid-beta hypothesis, caused by the 39-42 amino acid chain of the amyloid precursor protein. The aggregated form of the amyloid-beta oligomers attack the cell, effectively destroying it. The amyloid precursor protein is cleaved by BACE-1, which is composed of two proteases, β -secretase and γ -secretase. Amyloid beta proteins are

tions, like tight junctions in the neurons. This leads to cognitive loss in the AD brain.

Tau hypothesis

Tau proteins in the brain have six isoforms, ranging from 383-441 amino acids in length in the human adult brain (AnaSpec 2013). Tau can self-assemble into filaments (Bamburg and Bloom, 2009), which comprise neurofibrillary tangles. When self assembled, they “detach from the microtubules, thereby destabilizing microtubules and compromising axonal transport” (Hampel et al., 2010). They are also comprised of microtubule binding sites, which “form[s] the core structure” (Bamburg and Bloom, 2009) of the tau protein. These aggregated fibers are hyperphosphorylated, with at least 30 phosphorylation sites (Bamburg and Bloom 2009), which can compromise microtubules. “[P]hosphorylation at those sites might represent a switch from an intracellular environment that is non-permissive for tau self-assembly to one that is permissive” (Bamburg and Bloom 2009). This means that when tau is phosphorylated, it becomes active, and that when tau fails to self-assemble in absence of phosphorylation, it does not affect the cell. Tau also breaks down microtubules, which can af-

fect synaptic activity and axonal transport.

Tau also affects mitochondria in cells. It completely changed the structures of mitochondria, which become, “shorter organelles that undergo enlargement, cristae disorganization, and complete loss of inner structures and... decrease of mitochondrial mass” (Amadoro et al.). The abnormal mitochondria are then removed by lysosomes in affected cells (Amadoro et al., 2014). This leads to a reduction in the ATP produced in a cell, which slows down and impairs important cellular reactions. This can lead to cell death and cognitive loss.

Tau and AB interactions

Tau and amyloid beta proteins are both possible causes of AD in the brain, and many scientists believe that “AB-oligomers as well as...AB-extracts from human AD brain induce AD type of tau hyperphosphorylation in cultured neurons” (Bamburg and Bloom 2009). It has been shown that AB may induce hyperphosphorylation in cells, leading to tau-induced cell death, like releasing toxic tau-related proteins in the CSF (Hampel et al., 2010). This means that hyperphosphorylated tau in the AD brain may be caused by amyloid beta proteins in the cell. Amyloid beta proteins and tau are not mutually exclu-

sive of each other, but may interact to damage cells, as well as mitochondria. These are described as “roundish and pale with patchy matrices, sometimes swollen and suffering from the total disappearance of their inner cristae” (Amadoro et al., 2014), and also as “irregular and interrupted intermembrane space” (Amadoro et al., 2014). The destruction may result in cell death, since mitochondria would not be able to make enough ATP to perform normal life functions.

Glycogen synthase kinase 3

Glycogen synthase kinase 3 (GSK3) interacts with both tau and APP by modifying both of their substrates, promoting the phosphorylation of tau, and causing BACE-1 to use b-secretase to cleave APP. This promotes abnormal cleavage of APP, which is toxic for the brain. This means that GSK3 induces the hyperphosphorylation of tau, meaning that it can destabilize microtubules in the brain, and cleaving of toxic AB, causing cell and mitochondrial death in neurons, with “intracellular components are

released into the extracellular space” (Hernandez and Avila, 2008). This can potentially be toxic to the surrounding neurons, causing cognitive impairment, which not only affects the neuron affected specifically by AB, but the neurons surrounding it as well. GSK3 may also promote the overproduction of amyloid beta proteins, amplifying cell death in the AD brain.

Conclusion

Amyloid beta proteins and phosphorylated tau are both abnormal forms of normal proteins found in non-AD brains. Amyloid beta proteins are made of

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b-secretase cleaved APP instead of a-secretase APP, while tau is made of hyperphosphorylated 383-441 amino acid chain in the adult brain. Abnormal forms of these proteins attack the cell, including the mitochondria, causing neuronal death. An affect of this cell death is cognitive impairment, including memory loss, speech deficits, motor loss, dementia, and synaptic loss.

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Deja Vu by Matt Kenny (Junior)

One of the most common and intriguing phenomena in known as deja vu. This spontaneous feeling of having done, seen, heard, or felt something before has an unknown cause, despite its common occurrences. A major contributing factor to this is the fact that deja vu epi-

sodes will occur spontaneously, meaning that researchers do not have the ability to collect data on the brain activity that goes on during a genuine deja vu episode. Therefore, in order to understand the actions of the brain during an episode, the brain needs to be manipulated so that

a deja vu episode can be simulated.

There are two basic ways that can be used in order to simulate deja vu. The first is electrical brain stimulation through the use of pathologic methods. This method is quite consistent in its producing of deja vu in the participant. Using these

electrical stimulations, the activity that goes on during deja vu is simulated (Adachi 2010). While the data is quite generic and will not show any originality through different participants, electrical stimulation is quite consistent in its ability to work. However, there are many limits to the type of people that can be experimented on with this method. Only those with certain diseases of the brain, more specifically Temporal Lobe Epilepsy and Schizophrenia, are usually used as participants due to the fact that the stimulation is more consistent in creating a deja vu episode when participants have these brain abnormalities (Radwan-sky 1998). The reality for these participants is that

they will face harsh consequences for taking part in these experiments. This is one of the main reasons as to why it is so necessary for another way in which deja vu can be provoked to be found. For example, those with Temporal Lobe Epilepsy are very likely to experience a seizure after the deja vu episode which was caused by the electrical stimulation. This is the main reason as to why many scientists are searching for alternatives to electrical stimulation that still allows them to simulate deja vu (Brown 2008).

A second way in order to create a deja vu episode in any participant is through the process of creating false memory in the brain. This process is quite complicated and the steps for



creating this false memory need to be followed very strictly in order to be successful in creating a deja vu episode. This process involves showing one scene to a participant, and then showing them another scene that is only slightly altered. Because the second scene seems relatively similar to the original scene, the participant will feel as if they have seen this scene before, and will therefore experience deja vu (Brown 2008). This method leads to no known consequences for any participants, because in this case, the deja vu episode that the patient experiences is real. While electrical stimulation shows the activity that goes on in the brain during deja vu, the participant does not truly experience deja vu. In the method involving evoking false belief, the participant is simply being set up to experience deja vu. While the scene the participant is seeing is fake, the activity in their brain is not (Moulin 2010). Still the brain activity will be recorded and can be used, but the data will always be relatively the same, without some abnormalities that could easily be seen in true cases of deja vu.

The only problem with the method involving false belief is that it is nowhere near as consistent in creating a deja vu episode as electrical stimulation is. Therefore, many scientists

studying of deja vu will still decide to use pathology and electrical stimulation in order to create this deja vu activity (O'Connor 2012). In order to force scientists to turn to the use of of stage simulation rather than electrical stimulation, false belief needs to be perfected so that it has a higher consistency rate.

The opportunities that are potential when there is an ability to gather data on deja vu are immense. For example, deja vu may have a relation to seizures (Serendip 2009). This theory is supported by the fact that most people with diseases of the brain that are related to seizures will usually experience deja vu before a seizure occurs. Deja vu is seen, by some, as being a “mini-seizure” rather than being its own phenomenon. Therefore, being able to stop deja vu from happening could be very useful in learning how to stop seizures from happening in those with certain diseases, such as epilepsy and schizophrenia.

Many feel upset after going through the experience of deja vu. For that reason, it would be important to stop it at certain times, as some may even go as far as suffering from depression in rare cases, in which deja vu occurs very frequently. Understanding the neurological process behind deja vu would al-

low us to monitor and record data on it. If deja vu can be stopped, then the feeling of confusion and sadness as a reaction can be taken away. Also, a precursor of an oncoming seizure is a feeling of deja vu (Adachi 1999). There may be a possibility that deja vu is a cause of a seizure or generates it. Therefore, by knowing how deja vu arises and stops, there may be a way to stop or diminish temporal lobe epilepsy consequences. Also, another theory may be that the feeling of deja vu is simply the beginning of a seizure. Therefore, this theory would then go on to state that deja vu is simply a smaller version of a seizure. This would coincide with the fact that not everyone experiences deja vu, meaning that not all people experience seizures.

The most believed and trusted theory as to how deja vu works is the optical pathway delay theory, which states that there is a malfunction between the eyes actually seeing what is going on and the brain processing this into a memory. Another theory to the deja vu phenomenon is the “cellphone” theory.

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This theory states that when a person is focusing on one specific thing, they are still able to see the surrounding objects through their peripheral vision. The person seems conscious of the object being there without fully focusing on it. Then, when the person fully focuses on the object, they feel as if they remember it from somewhere else (Obringer 1998). This hypothesis is interesting because it poses the question: could it be possible for someone to provoke deja vu? If so, then scientists would be able to provoke it and would be able to generate models to study deja vu efficiently; Currently, deja

vu cannot be studied due to the fact that it only arises about once a year and the time in which this phenomenon will take place cannot really be predicted. Therefore, with this hypothesis, we could collect data about it, and further understand how this common phenomenon really does work. However, this theory is challenged constantly because of the fact that to this date no scientist has been able to provoke *deja vu*.

It is necessary for scientists to find another way to provoke *deja vu* because of all the potential findings that could come from that data. *Deja vu* has so many potential connections to many things, including Schizophrenia, Temporal Lobe Epilepsy, and much more. Without another way to provoke *deja vu*, scientists will struggle with many other fields. When considering one's brain stimulation and function, it is necessary to respect the effect that a person's spatial environment has on he/she. Spatial awareness can be defined as a person's ability to properly identify and comprehend information about an environment surrounding them. The most effective way to evaluate one's spatial awareness is through situation models. Situation models serve as reproductions of virtual spatial environments (Radvansky 2006). By

properly designing these situation models, it is possible through experimentation to stimulate certain parts of the brain that a specific spatial environment will have an effect on.

Humans may also have a difficulty in deciphering new information when entering new spatial environments: it may lower one's ability to properly comprehend the world around he or her. Gabriel Radvansky exemplifies this in his paper, titled *Walking Through Doorways Causes Forgetting*. In his experiment, Radvansky proves that the rapid and sudden entering of a completely new environment forces humans to have to identify a whole new set of preliminary and direct objects, causing them to forget previous ones (Radvansky 2006).

These situation models will go to great lengths in amplifying studies on *deja vu*. By designing the proper sequence of scenes and simulations, the potential is there to find a way to stimulate certain functions of the brain, so as to provoke *deja vu*. This alternative to less ethical provoking of *deja vu* has the ability to serve quite effectively.

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The Effects of the Wnt Calcium Pathway on Astrocytic Gliotransmission, by Robert McIlrath (Junior)

Abstract

The Wnt/ Ca^{2+} pathway is directly responsible for the amount of calcium ions available within astrocytes. Calcium ions are necessary for gliotransmission, which can cause changes in rates of neurogenesis in the hippocampal dentate gyrus, a region of the brain famous for continuing neurogenesis during adult life.

If calcium ions necessary for gliotransmission are acquired through the G-Protein dependent PLC enzyme of the Wnt/ Ca^{2+} pathway, then blocking the Fz receptors of the Wnt pathway using either Cer or FrzB will lead to a reduction in rates of gliotransmission. Also: if gliotransmission increases the rate of neurogenesis then, when stimulating/increasing gliotransmission rates, Brdu staining in the hippocampal dentate gyrus will reveal a comparative increase in adult young neurons compared to that un-stimulated gliotransmission rates.

Introduction

Astrocytes and gliotransmission

Astrocytes are cells within the brain long thought to be exclusively functioning as protective cells for neurons. In the past few years a concept, known as gliotransmission, has

gained popularity in the scientific community. Gliotransmission is a process by which neuroglia (a broad category of neuronal cells, containing astrocytes, oligodendrocytes, etc.) can communicate and send electrochemical signals to modify neuronal signaling (Nedergaard et al., 2003). Upon the P2Y1 receptor in the astrocyte receiving molecules of ATP vesicles containing glutamate release the gliotransmitter and enter the synaptic cleft, thereby causing the synapse to fire (Nedergaard et al., 2003).

Several recent studies have gone on to conclude that this gliotransmission process is integral in the differentiation of neuronal stem cells, determining whether they become oligodendrocytes, astrocytes, or neurons (Song et al., 2002). Going by these studies the addition of glutamatergic gliotransmission increases the likelihood for neuronal stem cells to differentiate into neurons. (Song et al., 2002) The region of the brain with the highest rate of young neurons in adulthood is in the hippocampal dentate gyrus.

The process of gliotransmission requires calcium ions in order to cause the release of gliotransmitters (Bezzi et al., 2001). The calcium ions are known to

cause regulated exocytosis of gliotransmitters that then go on to modulate synaptic activity (Zorec et al., 2011).

The WNT non-canonical Ca^{2+} pathway:

The non-canonical Wnt/ calcium pathway is used to deliver calcium ions inside the cell (Huelsen & Juergen, 2002). Wnts, secreted glycoproteins, bind to frizzled seven-transmembrane span receptors coupled to G proteins (Huelsen & Juergen, 2002). These glycoproteins, which are chains of simple sugars, bind to specific receptors on the astrocytes that trigger the Wnt/ Ca^{2+} pathway (Kohn & Moon, 2005). The activation of the Fz receptor causes Phospholipase C (PLC) to cleave membrane-bound phosphatidylinositol 4,5-bisphosphate (PIP2) into diacyl glycerol (DAG) and inositol 1,4,5-trisphosphate (IP3) (Kohn & Moon, 2005). IP3 proceeds to diffuse through the cytosol and cause calcium channels of the endoplasmic reticular membrane to release calcium ions (De, 2011).

These calcium ions normally lead to the activation of phosphatase calcineurin

causing the dephosphorylation of the transcription factor NFAT (Huelsen & Juergen, 2002). However the calcium ions could be involved in the calcium ion dependent action in gliotransmission, which is the hypothesis previously stated. In the past astrocytes have been reported to have the Wnt/ Ca^{2+} as their exclusive access to calcium ions (Agulhon et al., 2012). There is little supporting evidence claiming the existence of voltage gated calcium ion channels being present in astrocytes, and using G-Protein antagonists the calcium ion levels are severely depleted (Agulhon et al., 2012). The Fz proteins can be blocked via secreted factors such as Cerberus (Cer) and FrzB in order to block calcium from entering astrocytes (Huelsen & Juergen).



Neurological impacts:

Gliotransmission is said to have impacts on dendritic morphology in the adult young neurons it differentiates (Song et al., 2002). Other papers suggest that young neurons are integral to short term memory functionality and spatial relations (Nedergaard et al., 2003; Song et al., 2001; Zhao et al., 2008). Having a control over the amount of calcium ions available for gliotransmission may also be helpful in the treatment of psychological disorders (Agulhon et al., 2012). On top of this general modulation of synaptic transmission is shown to have impacts on sleep disorders and epilepsy (Zorec et al., 2012).

Quite simply this would greatly benefit the understanding of neurological development, especially in later life. Understanding the Wnt pathway's affect on the development of young neurons in the brain will ultimately provide new knowledge necessary when considering impacts of other experiments, as well as in the development of psychiatric drugs.

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Avian Facial Recognition and Discrimination, Jane Milcetic (Sophomore)

Until recently, the capabilities of the avian brain have been underestimated. Modern studies now focus on the astounding competence of the previously underrated species (Happ, 2013). Facial recognition, a skill thought to be specific to humans, seems to be possessed by some birds as well (Soto, 2011). Corvids, pigeons, and other fowl with high brain to body ratios have been able to identify human faces from one another, based on the bird's preference of discrimination (Bhatt, 1988; Bogale,

2010; Soto, 2011). Despite the building amount of research investigating the reason as to why birds can tell two people from one another based on their facial features, much is still unknown about the extent of this ability and its anatomical basis.

Birds have been discredited for their intelligence due to Ludwig Edinger's assumption in the 1800's that the avian brain had no gyri, leading other scientists to be disinterested with the supposedly primitive species (Happ, 2013). Edinger reasoned

that the wrinkle-free brain indicated that birds relied on instinct rather than processing thoughts and acting upon them (Happ, 2013). Past the barrier of supposition, the comparison of the human and the bird brain shown that they have similarities in functions, yet a different anatomical organization (Happ, 2013; University of California Television, 2008). The pallium exemplifies this, it being the outer layer of the brain in all vertebrates that has sections with functions specific to the species

(Weiner, 2013). Marzluff, Miyaoka, Minoshima and Cross found that when American crows (*Corvus brachyrhynchos*) viewed familiar human faces, their mesopallium and nidopallium activated (Marzluff, 2012). The mesopallium is used for imprinting, memorizing and recognizing birdsongs, while the nidopallium makes decisions (Weiner, 2013). Their discoveries shed more light on the enigmatic topic of avian neurology.

Posamentier and Abdi traced facial recognition in humans to the occipi-

tal gyri, the fusiform gyri, and the superior temporal sulcus (Posamentir, 2003). More developed in their definitions, they credit the occipital gyri with memorizing the basic form of facial features and their variations, the fusiform gyri with remembering unchanging facial features, and the superior temporal sulcus with perceiving emotion through observation of expression and motion. Shared by both the avian brain and the primate brain is the striatum, which is involved with learning and movement (Marzluff, 2012). The memory system sends signals between other components of the brain to store information, and interacts with the prefrontal cortex, the human version of the nidopallium (Weiner, 2013). Through the parallelism of the human and avian brain, more can be discovered about the latter, and even some about the former.

Bhatt, Wasserman, Reynolds and Knauss tested 8 feral pigeons on their ability to classify objects in the natural or manmade category (Bhatt, 1988). Using pictures of humans, cats, cars, and chairs that differed in their setting, visibility, orientation, et cetera, 8 birds were trained to identify an image in two ways. Since it has been proven that pigeons can memorize 200 slides if not more, Bhatt,

Wasserman, Reynolds and Knauss designed an experiment to test if their subjects were memorizing the 40 presented pictures, or relied on fitting the photograph into the four categories. Out of 2,000 slides, 40 images were chosen to be the familiar group, 10 for humans, 10 for cars, and so on. On uneven days, the familiar group was shown, while a new set of 40 pictures, each category represented by ten photos, was shown on the even days. The unfamiliar images were chosen from the remaining 1,960 slides. After 96 days, the pigeons finished their testing. While the subjects struggled with the non repeating photos, every bird's scores were above chance level. The highest scores in the repeating category were at 91%, and the lowest were at 20%. The highest scores in the non-repeating category were at 84%, and the lowest were at 19%. Since some pigeons succeeded at discriminating between images, though others lowered the statistics, as they lacked the competence to complete the task. The experiment was done another 3 times, each with the same subjects, though the 40 pictures in the familiar group were changed to ensure that the birds' scores were not affected by an odd image. Out of the 4 trials, the pigeons maintained

relatively consistent percentages, supposing that each subject had their own way of telling one category apart from another. It seemed that the birds were objectifying the humans, as they viewed still slides, and not moving pictures (Bhatt, 1988). While humans are able to recognize another person as a being and not an item, it is unclear how pigeons categorize people, not just pictures of them. A study by Marzluff, Miyaoka, Minoshima and Cross study leads to the idea that the parts of the avian brain may have undiscovered features that allows them to memorize the face of person and use it to their advantage (Marzluff, 2012).

Gibson, Wasserman, Goselin and Schyns tested 4 pigeons using colored photographs of 32 faces to see if the birds could categorize the stimuli, as well as what facial features and areas they used (Gibson, 2005). The subjects were trained to recognize either emotion or gender until they could categorize 32 plain faces and two partially occluded faces with 70% accuracy. Then, the same faces were blotted with "bubbles", computer-generated grey splotches that concealed parts of the face. The pigeons viewed 320 bubbled faces in a



block, and performed 7 blocks per day. Each of the 32 faces had its own percentage of how often the subjects correctly selected it, as did each bird. The final results showed the two in the emotional category scored 91% and 75%, while the gender category scored 71% and 85%. Faces that had the chin uncovered lead to those in the emotion group to select correctly, while a revealed mouth aided those in the gender group to determine the sex of the target. Bogale, Aoyama, and Sugita studied a similar concept with 6 jungle crows, evenly split between determining either if the photo face was male or female (Bogale, 2010). After each bird reached 80% correct with the regular photographs that showed men and women sans hair (eyebrows were kept) from the middle of the neck up, the images

were altered by making it colorless, contouring the face, or barring out certain features. When the pictures were greyed, the subjects gravitated towards the mouth and the eyes, indicating that the crows relied on color (particularly the hue of the pupils, and the jaw and chin region). These scientists suggest that crows may remember faces to obtain food, maintain dominance, and ward off unwelcome humans (Bogale, 2010). Urbanization is also pressing factor that forces birds to live with humans, and adapt to the new way of life. Since a bird must account for its predators, remembering a human face could save its life.

While the aforementioned studies delved into the foreign subject of the avian brain using methods to determine if birds can

use faces to discriminate between people, few have investigated how they do this. The use of MRI machines to view the areas of the bird brain is difficult, as the subjects constantly move, and sedatives hinder cognition. Even though chances of picturing the avian brain are slim, the use of motion incorporated in an experiment could improve the perception of the species. While most studies have used still photographs, thus creating the possibility that birds memorize the face as an object rather than a living thing (Bhatt, 1988). The use of repetitive training could result in birds telling one human apart from another (Bhatt 1988), though a mechanism in the avian brain could be responsible for their abilities.

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Using the Gut-Brain Axis to Treat Autism, by Jonathan Chung (Freshman)

Neuroscience is the study of the nervous system and how it develops, matures and maintains itself (Sukel 2011). Most commonly, it is associated with the approximately 86 billion nerve cells in the brain, which are called neurons (Sukel 2011). Neurons are primarily made up of two parts, which are the dendrites and the axons (Sukel 2011). In addition, these neurons contain thousands of synapses

each that are located at the end of dendrite and axon branches (Sukel 2011). The synapses allow the neurons to perform their function of transferring electrical and chemical messages from one neuron to another and through the body from a broader perspective (Sukel 2011). These synapses are similar to muscles in the sense that they become stronger if used and become weaker when not used (Sukel

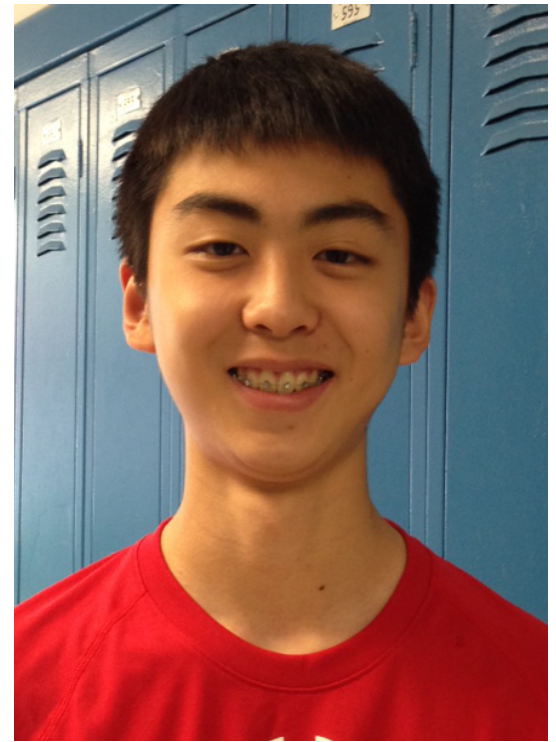
2011). This process of synapse strengthening and weakening is called potentiation and relies on synapse plasticity (Sukel 2011). Surprisingly as research continues, scientists are finding that there may be a link between the brain and gut microbiota. The radical idea that a gut-brain axis may exist continues to gain support as current research continues. A main contributor to beginning this entire field

of research was the fact that many mental illnesses such as autism, Rett syndrome and cerebral palsy have gastrointestinal issues as well (Hsiao et al; 2013). Children with these illnesses also have different gut microbiota composition from neurotypical children (Hsiao et al; 2013). So far, there are many explanations about how this axis works but none that are universally accepted. Suggestions

exist, such as the axis involving neuronal circuits, alterations in synaptic plasticity genes and the vagus nerve (Heijtz et al; 2011). However, it is likely that the gut-brain axis is a bidirectional homeostatic route of communication that uses neural, endocrine and immune pathways where the gastrointestinal tract provides the scaffold for these pathways (Cryan and Dinan 2012). This idea can provide the basis of new ways to prevent, diagnose, treat and potentially cure many neurological disorders (Kang et al; 2013). An emerging idea is to use probiotic therapy, where probiotics are live organisms that stimulate the growth of other microorganisms, usually ones that provide beneficial properties to the host (Cryan and Dinan 2012). Normally, probiotic therapy is used to treat issues and illnesses related to the gut, but could be used to treat neurological illnesses as well because of the growing support of the gut-brain axis (Cryan and Dinan 2012). For example, the probiotic of *Lactobacillus rhamnosus* decreased anxiety and despair-like behavior and reduced stress-induced increase of plasma corticosterone levels in mice (Cryan and Dinan 2012). In this particular case this most likely occurred because the probiotic altered the mRNA expression of

receptors in several brain regions (Cryan and Dinan 2012). Another example is the probiotic agent *Lactobacillus farciminis*, because it prevented intestinal barrier leakiness and also reversed psychological stress (Cryan and Dinan 2012). However, this is no surprise because chronic stress is known to disrupt the intestinal barrier making it leaky and is known to affect gut microbiota composition (Cryan and Dinan 2012). The gut microbiota composition is very important because it determines health since a rich and diverse gut is a healthy one (Kang et al; 2013). Irritable Bowel Syndrome patients support this claim because they have 25% fewer genes in the gut than healthy controls (Kang et al; 2013). A higher diversity of gut bacteria allows better microbial integrity and the ability to protect the human intestine from environmental stresses including the intake of pathogenic gut microbes (Kang et al; 2013). When the gut microbiota diversity is low, then the gut is more susceptible to pathogens, which can disrupt the gut-brain axis causing pathophysiological consequences (Cryan and Dinan 2012). The gut microbiota composition can be manipulated through diet, infection, disease and antibiotics (Cryan and Dinan 2012). This is both

a positive thing and a negative thing. On the positive side, this is what allows probiotic therapy and antibiotic treatment to work and cure diseases and restore gut microbiota diversity (Kang et al; 2013). Additionally, it allows specific diets such as a gluten and casein free diet to improve symptoms of autism (Finegold et al; 2002). However, on the other hand, this ability to change the diversity of the gut microbiota allows a western-style diet and overuse of antibiotics at an early age to contribute to problems (Kang et al; 2013). A strong piece of evidence supporting how gut microbiota influences the brain is how specific pathogen free mice (SPF) have altered behavior from germ free (GF) mice most likely due to their differences in gut microbiota (Heijtz et al; 2011). The germ free mice demonstrated increased motor activity and less anxiety-like behavior in comparison to specific pathogen free mice (Heijtz et al; 2011). However, when



these germ free mice were exposed to gut microbiota in early life they displayed a behavioral phenotype similar to the specific pathogen free mice (Heijtz et al; 2011). This scenario is extremely similar to how the maternal immune activation (MiA) model works. In the MIA model, infected mothers have a higher chance of having offspring with symptoms of autism (Hasio et al; 2013). Both instances occur because of developmental programming which says that early life environmental influences impact an organism's later development, structure and function (Heijtz et al; 2011).

Autism is a neurodevelopmental disease that is closely related to the gut-brain axis. It is diagnosed when people show signs in their behavior such as

deficits in social interaction and language. Interestingly, at the onset of autism gastrointestinal symptoms arise (Finegold et al; 2002). These symptoms are altered gastrointestinal motility and increased intestinal permeability (Hsiao et al; 2013). Additionally, significant alterations in the upper and lower intestinal flora of children occur at the onset of autism (Finegold et al; 2002). The alterations in the intestinal flora cause the intestinal gut microbiota composition of children with autism to be different from neurotypical children (Cryan and Dinan 2012). This change in composition is a loss of diversity proven by autistic children having lower levels of a group of distinctive bacterial genera (Kang et al; 2013). This less diverse and rich gut has negative correlation with gastrointestinal severity reinforcing the idea that a healthy gut is a diverse one (Kang et al; 2013). Specifically, lower levels of the *Prevotella* genera were found to be most significant (Kang et al; 2013). It happens that the *Prevotella* genus is important even outside of its significance in it being the difference in autistic gut microbiota composition. The *Prevotella* genus determines the gut-microbiome profile and was proposed as one of the three main microbes representing the human gut microbiome (Kang et al; 2013). In addition, the *Prevotella* species have essential genes for the biosynthesis of vitamin B1, which was reported to alleviate Autism Spectrum Disorder symptoms (Kang et al; 2013). Within the *Prevotella* genus, is *Prevotella copri*, which was found to be the most significant species in the *Prevotella* genus for differences in the gut microbiota composition of autistic children (Kang et al; 2013). When there is a loss of *Prevotella copri*-like operational taxonomic units in autistic samples it could result in a major reconfiguration and reduced complexity in the intestinal microbial ecosystem (Kang et al; 2013). This loss could also contribute to why the community-wide interrelationship of gut microbiota is altered in autistic children (Kang et al; 2013). Although not proven it is a possibility because neurotypical children have a negative correlation between *Prevotella* levels and *Enterobacteriaceae* while autistic children had none (Kang et al; 2013). There are ideas that these decreases in *Prevotella* and certain levels of species in general may be due to diet patterns (Kang et al; 2013). For example, a high carbohydrate-based diet shifts the intestinal microbiota towards the *Prevotella*-rich enterotype (Kang et al; 2013). Another theory for the causes of autism from the gut microbiota is different levels of serum metabolites (Hsiao et al; 2013). This is because the offspring of the MIA model had symptoms of autism including deficiencies in pre-pulse inhibition, deficiencies in communication and repetitive behavior but also had elevated levels of certain serum metabolites (Hsiao et al; 2013). There were a plethora of different levels of serum metabolites between the autistic mice and normal mice (Hsiao et al; 2013). The metabolites believed to be most important in the cause of these Autism Spectrum Disorder symptoms were 4-ethylphenylsulfate and indolepyruvate (Hsiao et al; 2013). Both of these had significantly increased levels in MIA offspring such as 4-ethylphenylsulfate, which had a 46-fold increase (Hsiao et al; 2013). When naïve mice were given these metabolites independently they expressed elevated or reduced amounts of certain behavior (Hsiao et al; 2013). For example, 4-ethylphenylsulfate caused mice to express increased anxiety-like behavior (Hsiao et al; 2013). However, some behaviors were not present when 4-ethylphenylsulfate or indolepyruvate were given suggesting that these metabolites in combination with others cause ASD behavioral symptoms as well (Hsiao et al; 2013). When the MIA offspring were given *Bacteroides fragilis* in probiotic therapy, metabolite levels were reduced to normal including 4-ethylphenylsulfate and indolepyruvate (Hsiao et al; 2013). These same mice also showed that behavioral problems were ameliorated including communicative behavior, repetitive behavior and anxiety-like behavior after probiotic therapy (Hsiao et al; 2013). Intestinal barrier permeability integrity also increased meaning that fewer metabolites were leaked into the blood stream (Hsiao et al; 2013). This suggests that behavioral symptoms of the MIA model may be caused by extra metabolites leaking into the blood stream and circulating to other parts of the body (Hsiao et al; 2013). The prevalence of autism in children exceeds juvenile diabetes, childhood cancer and pediatric AIDS combined (Kang et al; 2013). Scarcely, there is still a rapidly growing rate of autism in the U.S. with 1 out of 88 children with autism as of 2008 (Hsiao et al; 2013). Fortunately, at the same time, there are new ideas that are rapidly emerging as well, including the gut-brain axis. This could serve as the foundation for many potential cures such

as probiotic therapy for autism and neurological illnesses in general. We also know that elevated serum metabolite levels and decreased gut diversity may be the causes of autism. On the other hand, we do not know exactly how these ideas, although clearly shown, work. There are many questions to be posed on this upcoming topic such as how these two different ideas may be connected. Personally, I wonder how these gastrointestinal issues affect the brain, and what is the main gastrointestinal cause of autism. Additionally, could all behaviors be traced to a

microorganism in the gut? If so, perhaps specific behaviors could be increased or muted by probiotic therapy. Finally, because of the rising prevalence of autism, I wonder what is the role of new technologies and culture, such as increased use of antibiotics, increased use of gut altering substances and a westernized diet?

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The Morbillivirus Epidemics in Dolphins and Marine Mammals, by Isabella Brizzi (Freshman).

Viruses spread very easily, through air, through water, through touch, and sometimes just through being in a largely populated area, can spread viruses. Sometimes, the spreading of viruses can cause detrimental devastation to a species' population. Viruses do not only spread this rapidly through not only mammals, but all organisms. One example of a virus, that specially effects only animals is called an epizootic. The Dolphin Morbillivirus (DMV) is a ubiquitous, deadly epizootic that is sweeping through the east coast. DMV belongs to the Paramyxoviridae family, a

strand of RNA viruses that cause human viruses and animal epizootics (Rima, B. K. & Duprex 2006). The Morbillivirus is also extremely similar to the human virus the measles. Both the Morbillivirus and the measles take a very similar course in weakening the immune system, and therefore both viruses have similar effects. Within the last 10 years, the Morbillivirus has appeared in 5 large and devastating epidemics (Kennedy 1998). The Morbillivirus has not only been seen off of the east coast of the United States, but also on the other side of the world, off of the

Mediterranean. In 1990-1992, off of the coast of the Mediterranean, there was a large epidemic that affected the striped dolphins (*Stenella coeruleoalba*). In 1993-1994 the Morbillivirus effected the bottle nose dolphins off the coast of the Gulf of Mexico. In 1987-1988 the Morbillivirus affected the bottle nose dolphins off the east coast

of the United States. The outbreaks of the Morbillivirus navigated around



the world, ending up off the east coast of the United States. The most recent outbreak occurred this past summer, of 2013, where hundreds of dolphins washed up along the shore lines. The dolphins washed up usually dead and beached on the shore. Some of the dead dolphins were already beginning to show signs of decomposition.

The Dolphin Morbillivirus not only kills dolphins and weakens their immune system, but it also affects them physically as well. The Dolphin Morbillivirus causes skin lesions, brain infections, pneumonia, and other illnesses and diseases that are caused by the dolphin's weakened immune system. All ages and genders are effected by the Dolphin Morbillivirus, but studies show that younger dolphins seem to be more severely affected by the disease. This happens in places where the epizootic has already occurred, because the younger dolphins have never been exposed to the virus and therefore are not immune the virus like some of the older dolphins are (Raja and Banyard 2008).

Even though the dolphin Morbillivirus is known to have large outbreaks within the various species of dolphins, it also has been discovered in various species of whales. As of November, 2013, 4

whales washed up along the east coast, between the shores of Massachusetts and Georgia, dead, infected by the morbillivirus (Daily News Reporter 2013). The whale species that was infected included 3 humpback whales, and one endangered pygmy sperm whale (Daily News Reporter 2013).

Like most viruses, after time doctors and scientists are able to come up with a cure or prevention to the specific virus. Scientists are still unsure of how to cease the mass dolphin deaths (Pittman 2013). Unlike a human virus, scientists cannot just create a vaccination for the dolphins, because it would be nearly impossible to prevent the species from contracting this specific epizootic.

The mass outbreaks seem to move around the world. It seems as though one moment the virus is taking its course off the coast of the Mediterranean, and the next moment it seems as though it is creating havoc off the shores of New Jersey. How is it when the dolphins' migration patterns do not associate with one another, both species of dolphin are still effected by the same epizootic, on opposite sides of the world? The answer may not be laying within the virus itself, but the things that cause this virus. Possibly the answer lays within the environmental

factors, which each individual species of dolphin inhabits.

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Parents

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Administrators, Teachers and Staff.

- **Mr. Joseph Hochreiter**, Superintendent
- **Dr. Alice Gottlieb**, Assistant Superintendent for Curriculum.
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- **Ms. Beth Gruber**, HH-CEF.
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Members of the School Institutional Review Board:

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

- **James Mackin**, Principal.
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